EFFECT OF COLD STRESS DURING TRANSPORTATION ON POST-MORTEM METABOLISM AND CHICKEN MEAT QUALITY

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ABSTRACT

Several studies were designed to investigate effect of cold winter transportation on broiler meat quality and physiological parameters. The first study assessed the effect of microclimate environment during winter transportation (-27 to +11ºC) from farm to the processing plant (3-4 h) on core body temperature (CBT) and breast meat quality parameters of 522 broiler chickens using an actively ventilated transport vehicle. Temperatures below freezing during transportation resulted in significantly ($P < 0.05$) higher ultimate pH (pH_u) and darker color breast meat, which led to 8% incidence of dark, firm and dry (DFD) breast meat (pH > 6.1; L*< 46).

The effects of extreme cold conditions (-18 to -4ºC) along with age (5 and 6 wk), gender and lairage (0 and 2 h) on physiology parameters, breast muscle metabolites and meat quality of 360 birds using a simulated transport system were investigated. Five and 6 wk birds exposed to temperatures below -8 and -14ºC, respectively, showed significantly ($P < 0.05$) lower CBT and blood glucose and higher live shrink with breast meat that was darker in color, higher in pH_u, water holding and water binding capacity compared to breast meat of birds exposed to warmer temperatures. A high incidence of DFD breast meat (> 50%) was observed for 5 and 6 wk birds exposed to temperatures below -8 and -14ºC respectively. Lairage following acute cold exposure caused an increase of up to 20% in DFD breast meat following exposure of the birds to temperatures below -8ºC.

Characteristics of cold-induced DFD breast meat were investigated in broiler chickens. DFD breast meat was significantly darker, redder, and less yellow in color and had higher pH, water binding capacity, processing cook yield and lower thaw and cook loss compared to normal meat. However, no significant difference in initial energy reserves was observed between DFD and normal breast meat.

Effect of extreme cold-stress (-15 to -9ºC) was further assessed on thigh meat quality and incidence of DFD from 160 male broilers. A greater impact of cold stress was observed on muscle metabolites and pH of thigh compared to breast meat, with 85% DFD incidence in thigh compared to 42% in breast meat of cold-stressed birds.

Biochemical basis of cold-induced DFD breast and thigh meat was investigated by measuring muscle metabolites at different times post-mortem. Lack of energy reserves at slaughter clearly explained the incidence of DFD thigh meat, but other factors might contribute to DFD breast meat aside from glycogen reserve at slaughter. However, the factors leading to DFD breast meat still remain unresolved and association of post-mortem enzyme activity and development of DFD breast meat merits further investigation.

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1. INTRODUCTION

The twelve h prior to slaughter is a time of severe activity that can be extremely stressful to the animal. Pre-slaughter stress including handling, loading and unloading, transportation, and feed deprivation can influence animal welfare and meat quality parameters such as color, texture, and related protein functionality, with negative effects on consumer acceptability and processing functionality of further processed products. Muscle is an active tissue and it responds to its environment before, during, and after death of the animal. Therefore factors involved in all these procedures could affect subsequent quality of meat and meat products. Transportation is an essential component of the poultry industry, but places enormous stress on birds because they have little space for behavioural thermoregulation. In particular, exposure to harsh weather conditions might cause suffering or death to birds from heat or cold stress (Hunter et al., 2001). Several researchers have studied the effect of handling (Nijdam et al., 2005b), transportation (Freeman et al., 1984; Warriss et al., 1993; Northcutt et al., 1994; Owens and Sams, 2000), crating duration (Kannan et al., 1997), pre-slaughter heat stress (Wood and Richards, 1975; Babji et al., 1982; Mckee and Sams, 1997; Sandercock et al., 2001), cold stress (Lee et al., 1976; Froning, 1978: Babji et al., 1982; Holm and Fletcher, 1997) and feed withdrawal (Warriss et al., 1993; Smith et al., 2002; Nijdam et al, 2005a; Lin et al., 2007) on welfare and meat quality parameters of poultry breast meat. The effect of heat stress has been extensively studied in poultry, however, there are only a few studies available on the effect of moderate cold conditions prior to slaughter on broiler chicken breast meat quality, and therefore further study is required that could be applied to regions in the world with extremely cold winter conditions.

In addition factors such as genetics, growth rate, age of the birds and gender might affect quality of the meat. Several studies have looked at effect of genetic selection (Berri et al., 2001; Fernandez et al., 2001; Le Bihan-Duval et al., 2001; Debut et al., 2003), age (Sandercock et al., 2001; Smith et al., 2002; Anadon, 2002; Bianchi et

al., 2006, 2007), gender (Anadon, 2002) and slaughtering procedures (Savenije et al., 2002; Nijdam et al., 2005b; Debut et al., 2005) in poultry. However, the reports of these factors on bird physiology and meat quality parameters of broiler and turkey breast meat are contradictory. Therefore there is no conclusive report on the effect of age or gender of the birds at slaughter on physiological parameters and meat quality.

Poultry is the only species with distinct differences in meat color by having white breast meat and red thigh and leg meat, due to significant differences in muscle fiber types which causes metabolic differences in post-mortem conversion of muscle to meat. Red fibers are high in myoglobin, they contract more slowly but have the capacity to operate for a longer period of time; therefore they are equipped with a higher number and larger mitochondria than white fibers (Barbut, 2002). In addition the higher lipid content allows the fibers to contract for a longer period of time. White fibers, also known as glycolytic fibers, on the other hand have less myoglobin content, less mitochondria with smaller size, a high content of glycogen and are able to perform in either the presence or absence of oxygen (Barbut, 2002). The majority of the studies have been conducted on white breast meat and only a few studies have investigated effect of pre-slaughter factors on quality of thigh meat (Debut et al., 2003; Kannan et al., 1997). Hence, further research is required to investigate quality properties of red thigh meat and compare it with white breast meat in broiler and turkey.

Two main types of poultry meat quality defects are known as pale, soft and exudative (PSE) and dark, firm and dry (DFD) meat, which can develop as a result of stress prior to slaughter. PSE incidence in poultry breast meat has been extensively studied (McKee and Sams, 1997; Holm and Fletcher, 1997; Owens and Sams, 2000), and it is associated with heat stress prior to slaughter, which causes a fast drop in pH while muscle temperature is still high immediately after slaughter resulting in meat with low ultimate pH (pH_u), light color and reduced water holding capacity. The DFD defect, on the other hand results from stress prior to slaughter, which causes depletion of muscle glycogen resulting in higher post-mortem muscle pH due to prevention of glycolysis by eliminating its substrate. DFD meat is dark in color, firm in texture and has a dry appearance or high water-holding capacity (Owens and Sams, 2000).

However, only a few studies have investigated the basis of DFD breast meat in poultry. No study is available on thigh meat quality defects in poultry. These defects could cause huge losses to the poultry industry, therefore understanding of the biochemistry behind development of these defects is essential to decrease or prevent their occurrence in the poultry meat industry. In order to fulfill these requirements, several studies were conducted.

Study I

Hypothesis: Cold winter transport affects bird physiology and subsequent meat quality.

Objectives: To examine the effect of cold exposure during transport of broilers from the farm to the processing plant on physiological responses and subsequent meat quality parameters, using an actively ventilated transport vehicle during all seasons with focus on Saskatchewan winters (0 to -40ºC).

Study II

Hypothesis: Severe cold exposure prior to slaughter affects bird physiology, muscle metabolites, meat quality and incidence of DFD breast meat that might be different for male and females at different ages or given different length of rest prior to slaughter

Objectives: To assess effect of extreme cold temperatures using a simulated transport system on bird physiology responses, muscle metabolites and meat quality factors, considering effects of age, gender and lairage time prior to slaughter.

Study III

Hypothesis: Cold-induced DFD defect in breast meat is a result of low glycogen reserves at slaughter.

Objectives: To investigate occurrence of cold-induced DFD breast meat and its characteristics.

Study IV

Hypothesis: Thigh muscle might respond differently to cold-stress compared to breast muscle.

Objectives: To assess the effect of extreme cold temperatures during simulated transport on thigh meat quality and incidence of DFD and compare it with breast meat.

Study V

Hypothesis: The basis for incidence of DFD meat could be different between breast and thigh muscles. Activity of AMP-activated protein kinase (AMPK) might be involved in development of DFD breast meat.

Objectives: To measure muscle energy reserves at different times post-slaughter on breast and thigh muscles to investigate biochemistry behind DFD development in the two muscles. To investigate the activity of AMPK in relation to DFD development in breast meat.

2. LITERATURE REVIEW

2.1 Chicken Meat Quality

Meat quality can be described as the overall characteristics of meat including its physical, chemical, biochemical, microbial, technological, sensory, nutritional and cooking properties. Some properties are important to consumers including appearance (color), texture, juiciness, tenderness, odor and flavor, which influence their judgement of the meat quality prior or after purchasing the meat, whereas properties such as water holding capacity, shear force, drip loss, cook loss, pH, shelf life, protein solubility, and fat binding capacity are essential for processors of value added meat products (Allen et al., 1998). However, the poultry grading system used worldwide is based on visual attributes such as presence or absence of carcass defects, bruises, missing parts, and skin tears without paying attention to the functional properties of meat, which are extremely important for the further processing industry (Barbut, 1996). Meat quality is usually assessed by measuring its pH, color and water holding capacity (WHC), since these are three main attributes for fresh and further processed products.

2.1.1 Meat pH

Meat pH by far is the most important parameter contributing to meat quality and protein functionality. Muscle pH is associated with numerous other meat quality attributes such as color, WHC, tenderness, juiciness and microbial stability (shelf-life) due to its effect on protein structure and hydration properties. The initial rate of pH decline and ultimate pH (pH_u) of meat, reached at 5-6 h post-mortem, are the two principal causes of variation in the quality of chicken meat. A strong relationship between pH_u and lightness (L* value) of the breast meat in chicken (Allen et al., 1997, Barbut, 1997; Qiao et al., 2001; Anadon, 2002; Barbut et al., 2005) and turkey (Owens et al, 2000) is reported. Furthermore, pH_u and L* are strongly linked and correlated with drip loss of the raw meat (Le Bihan-Duval et al., 2001) and water-holding capacity (Le

Bihan-Duval et al., 2001) and cooking yield (Debut et al., 2003). As pH of meat drops to the isoelectric point (where positive and negative charges are equal on proteins), solubility and water binding capacity are minimal, because firstly there is no net charge on the proteins to bind to water molecules and secondly there is less space for water within myofibrils due to increased affinity within myofibrils. Isoelectric point of muscle myofibrillar proteins is close to a pH of 5.5 (Swatland, 1994). However, Srihari et al. (1981) have reported different isoelectric points for various components of myofibrillar proteins, with actin having pI from 5.5 to 5.8; myosin heavy chain from 6.3-7.3 and myosin light chain from 4.8 to 5.6.

Understanding the relationship between pH_u and L* value could be useful for the quality differentiation of poultry meat, making it possible to use this meat adequately by the industry. High final or "ultimate" pH produces dark, firm and dry (DFD) meat with high WHC, but a poor storage quality due to high moisture content and a faster rate of off-odor production and accelerated microbiological growth (Allen et al., 1998; Le Bihan-Duval, 2004). On the contrary, a fast drop in pH post-mortem and a low final pH produces meat with an improved shelf life but a pale color, soft texture and reduced WHC (PSE; pale soft, exudative), which will be discussed in more detail later.

2.1.2 Meat color

Meat color is an important quality factor and has a great influence on consumer acceptability and purchase decisions, because consumers relate meat color to its freshness and overall quality. Poultry is the only species with meat that shows marked differences in color due to muscle biochemistry and histology. These meats are classified as either white (breast meat with pale pink color) or dark (thigh and leg meat with dark red color) meat. White and red fibers have different characteristics, which will be discussed later, but the main noticeable difference to consumers is their color, which is a result of myoglobin pigment concentration. Changes in breast muscle color is more noticeable because of its natural light color and because breast comprises a higher proportion of the carcass (Anadon, 2002). Color variation within a package of skinless

fillets could cause rejection of the entire package and a great loss to the industry, because consumers are more sensitive to color variations than to absolute color (Fletcher, 2002).

Meat color varies based on the concentration of myoglobin and hemoglobin (major pigments imparting meat color), pigment chemical state, or the way light is reflected off the meat (Froning, 1995). Hemoglobin is found in red blood cells and its concentration in meat depends on the efficiency of bleeding during slaughter (Swatland, 1994). Myoglobin is a soluble protein formed from a single polypeptide chain, which surrounds an oxygen-carrying heme group composed of an atom of iron and a porphyrin ring. The primary function of myoglobin is to transport oxygen within the muscle fiber (Swatland, 1994). Concentration of myoglobin can be affected by factors such as species, age, sex and genotype (Barbut, 2002), location of the muscle, and muscle activity (Barbut, 2002). Fiber type and myoglobin content have strong effects on meat color, since the basic difference in meat color is due to relative amounts of white and red fibers. Post-mortem temperature and pH play crucial roles on the extent of protein denaturation and physical appearance of meat (Lawrie, 1998). Light scattering from a muscle surface is directly proportional to the extent of protein denaturation, where at a pH $\geq$ 6.0 that protein denaturation is minimal, water molecules are tightly bound, causing more light to be absorbed by the muscle, and the meat appears darker in color. Whereas, at pH $\leq$ 6.0, protein denaturation is higher, light scattering increases, and muscle becomes opaque. Changes in light scattering affect meat lightness (L*), but has a minimal effect on meat redness (a*) and yellowness (b*) that is opposite to that caused by heme pigment concentration (Barbut, 1997; Swatland, 1994).

Color of meat can be evaluated using different systems, the most popular of which is the CIE LAB, defined by the CIE (1978). Another frequently used method is Hunter L, a, b solids scale. In the CIE LAB, the L* value is an expression of the lightness of the surface ranging from 0-100 (black to white), a* value indicates red, ranging from negative to positive (green to red), and b* value also ranging from negative to positive, which stands for blue to yellow (Barbut, 2002). The time at which

L* value is measured could affect reading values of L*, since it is reported that meat lightness increases significantly over time post-mortem (McCurdy et al., 1996).

Meat color have been related to other meat quality parameters and functional properties of meat in a number of studies (Barbut, 1997; Bianchi and Fletcher, 2002; Bianchi et al., 2004, 2005, 2006, 2007; Fletcher et al., 2000; Owens et al., 2000; Qiao et al., 2001; Van Laack, 2000), showing that L* measurements can be used as an indicator of poultry breast meat quality for further processed products as well as poultry meat defects including PSE (pale, soft, exudative) and DFD (dark, firm, dry) conditions. Dark broiler breast fillets are reported to have significantly lower lightness values (L*), higher redness values (a*), and lower yellowness values (b*) than light broiler breast fillets (Allen et al., 1997, 1998; Petracci et al., 2004; Barbut et al., 2005; Bianchi et al., 2007). A wide range in lightness (L*) of breast meat has been reported by different researchers ranging from; 35.3 to 55.6 (Barbut, 1997), 42.0 to 71.0 (Woelfel et al., 2002), 45.0 to 67.0 (Wilkins et al., 2000), 47.7 to 66.5 (average of 56.7) (Anadon, 2002), 40.0 to 66.0 (Petracci et al. 2004), and 41.0 to 56.0 (Barbut, 1998; Lesiow et al., 2007). This wide variation in L* values are reflective of the wide distribution of muscle pH values at 24 h post-mortem because these traits are inversely correlated. Variation in L* value depends on many factors including genetics, age, sex, flock, nutrition, season of the year, etc that will be discussed later in this chapter.

2.1.3 Water holding capacity

WHC is one of the most important functional properties of meat and an important quality attribute for both processors and consumers. The ability of meat to hold water helps with tenderness, juiciness, firmness and appearance of the meat, leading to an improvement in quality and economic value. WHC of meat can be categorized as water binding potential (WBP), expressible moisture and free drip, each having different applications. For instance, WBP is defined as the ability of the muscle proteins to retain excess water and under the influence of external forces; therefore it represents the maximum amount of water that muscle proteins can retain under the established condition (Swatland, 1994). Expressible moisture represents the amount of

water that can be expelled out of the meat by use of force, and free drip is the amount of water lost from the meat without any force other than gravity (Swatland, 1994), which is important for retail display and consumer acceptability of tray packed meat.

Most of the water (88 to 95%) inside the muscle is held within intracellular spaces between actin and myosin filaments, and only a small portion (5 to 12%) is located between the myofibrils (Offer and Knight, 1988). Several factors such as pH, sarcomere length, ionic strength, osmotic pressure and development of rigor mortis influence WHC (Offer and Knight, 1988). After animal death, lactic acid is produced and pH declines causing a reduction in water binding ability of the meat due to protein denaturation, loss of protein solubility and therefore reduction of reactive groups available for water binding on muscle proteins (Offer and Knight, 1988). When pH decreases to values close to the isoelectric point of proteins, water binding ability of the proteins is impaired, thick and thin filaments move closer together, myofibrils shrink, and the volume of sarcoplasm increases. Eventually, muscle fibers deplete all their ATP, their membranes no longer confine the cell water, and fluid is lost from the muscle fiber that may contribute to the exudate lost from the meat (Swatland, 1994).

2.2 Factors Affecting Meat Quality

All the procedures from the time bird is prepared for transfer to the slaughter plant, handling and transport, processing procedure and post slaughter practices affect the subsequent quality of the meat and meat products. Some of these pre-slaughter factors, including feed withdrawal, transportation and environmental changes as well as conversion of muscle to meat and post slaughter temperature will be discussed here. In addition factors such as genetics, gender and age at slaughter and muscle fiber type will be discussed since they also play important roles in the subsequent meat quality.

2.2.1 Feed withdrawal prior to slaughter

Feed withdrawal periods of 9-10 hours prior to slaughter are common in the poultry industry (Savenije et al., 2002), however, longer periods have been reported as

well (Nijdam et al., 2004). Feed is usually withdrawn for several hours prior to catching in order to reduce carcass contamination (Savenije et al., 2002). The process of catching and loading, along with transportation duration and time spent at the slaughter house prior to processing (lairage) could take anywhere from 5 to 15 h (Nijdam et al., 2004). Therefore, total feed withdrawal time on average could take from about 12 h to maximum of 33 h in the worst case scenario. The major adverse effect of feed withdrawal is the loss in body weight of between 0.22 and 0.56% / h starting after 4-6 h of fasting (Lyon et al., 1991; Knowles et al., 1995; Warriss et al., 2004). Nijdam et al. (2005a) showed a decrease of 0.42% / h for birds after 10 h feed withdrawal period and 1.5 h transport. Broilers that had full access to feed until the moment of transport lost 0.30% / h less body weight. A large amount of the body weight reduction is due to the emptiness of the gastrointestinal tract (Warriss et al., 2004), as well as a decrease in the weight of edible parts due to dehydration and losses of fat and protein (Knowles et al., 1995). Knowles et al. (1995) reported a 10% drop in live weight (0.43% / h) as a result of 24 h food and water deprivation, of which 41% was loss in carcass weight. However, Warriss et al. (1993), and Smith et al. (2002) did not find any effect of feed withdrawal times of 1-10 h and 0-8 h, respectively, on live and carcass weights.

Effects of feed withdrawal on blood glucose, liver and muscle glycogen have been studied. A negligible amount of glycogen has been reported in the liver of feed-deprived birds after several hours of feed withdrawal (Mellor et al., 1958; Nijdam et al., 2005a; Savenije et al., 2002). However, the reports on effect of feed withdrawal on blood glucose, muscle glycogen and meat quality are contradictory. It was proposed that changes in body weight, liver weight and liver glycogen content can influence the subsequent meat quality by affecting muscle glycogen content at slaughter, which affects the rate of rigor as well as the pH_u of the meat (Fletcher, 2002). Savenije et al. (2002) did not find any effect of feed deprivation and transport for short periods (5 h of feed withdrawal followed by 1.5 h of transport) on blood glucose or muscle lactate and glycogen levels or breast meat pH of chickens. Ngoka et al. (1982) also showed that feeding or feed withdrawal did not affect turkey breast muscle glycogen, initial pH, ash, fat, protein or breast meat quality attributes such as color, cooking loss, and thaw loss values. Warriss et al. (1993) also did not report any effect of feed deprivation on breast

muscle glycogen content. In addition, Kotula and Wang (1994) did not report any effect of fasting on the color of chicken breast meat. On the other hand, a significant increase in pH_u of the breast meat was reported by Warriss et al. (1993) as a result of feed withdrawal. Nijdam et al. (2005a) reported lower glucose, and lactate concentrations for broilers having no access to feed before transport compared to those who had access to feed and water right before transport. Smith et al. (2002) also reported a significant effect of diet and feed withdrawal on raw breast meat color, with increased lightness (46 to 49), decreased redness (4.1 to 3.1), and increased yellowness (2.8 to 3.7) as a result of feed withdrawal, with no effect on carcass or fillet weight. A greater effect of feed withdrawal on glycogen content of chicken "red muscle" rather than "white muscle" was reported by some authors (Kannan et al., 1997). Therefore, effect of fasting on initial post-mortem muscle glycogen levels and pH, as well as color and other quality parameters of the breast meat, ranges from no to some effect; but its adverse effect on live shrink from an economical stand point is obvious and is not favourable to the industry.

2.2.2 Effect of heat and cold stress on bird physiology and meat quality

Heat and cold stresses are two major contributors to both death and overall transportation stress in broilers. Post-mortem assessment of death on arrival has revealed that 40% of birds die from thermal stress due to failures in thermoregulation, therefore understanding the principles of heat balance in poultry is necessary to understand regulation of thermogenesis in birds. Chickens are homeothermic, meaning they can maintain their body temperature at 41-42ºC under ambient temperatures, with body temperature of 45-47ºC as the critical upper limit and 19-22ºC as the lower critical limits for males and a few degrees higher for females (Nicole and Scott, 1990). Birds need to balance the heat produced by metabolism with the heat lost to the environment. The thermoneutral zone for any homeothermic is the range of air temperature wherein the animal is able to regulate heat loss in order to match the heat production through metabolism. When environmental temperature rises above the upper critical temperature, heat production cannot be reduced effectively in the short term and

hyperthermia could occur (Webster et al., 1993). Below the lower critical temperature, heat production must be increased by cold thermogenesis to maintain core body temperature. With hypothermia, the core body temperature of bird drops below the lower critical temperature as a result of exposure to cold ambient temperatures especially at high wind speed (Nicole and Scott, 1990). The fat located under skin and feathers are the main factors in providing insulation for birds, thus poorly feathered, wet or dirty birds lose heat faster. Wind can also reduce the insulation effect by penetrating into feathers. The rate and amount of heat loss through respiration and skin partially depends on the water vapour density gradients (i.e. the degree of air saturation around the bird) and physiological response of the birds (Nicole and Scott, 1990; Webster et al., 1993; Weeks and Nicole, 2000).

Environmental conditions during transport and holding of poultry have been shown to affect bird welfare and subsequent meat quality. Holding conditions prior to slaughter might dramatically affect live bird shrink (3.2, 3.9 and 5.7% for temperatures of 25, 29.5, and 34ºC respectively) and apparent yields (Petracci et al., 2001). The increase in live shrink with increase in holding temperature prior to slaughter was also reported by Holm and Fletcher (1997). This loss in live weight is not desirable from both welfare and economic points of view and could cause considerable loss to the poultry industry. In addition, pre-slaughter environmental stress could affect post-mortem metabolism and resultant meat quality.

Heat stress has been reported to accelerate rate and extent of rigor mortis development, post-mortem glycolysis, and biochemical changes in the muscle, causing undesirable changes in meat characteristics similar to the PSE condition reported in pork (McKee and Sams, 1997, 1998; Sams, 1999; Sandercock et al., 2001; Wood and Richards, 1975). However, no study has looked at the effect of cold stress on post-mortem metabolism.

There are contradictory reports on the effect of environmental stress on poultry breast and thigh meat quality. The majority of research studies have shown a decline in pH and WHC of the breast meat as shown by an increase in drip loss, and cooking loss and an increase in lightness of broilers and turkeys as a result of acute or seasonal heat

stress (Babji et al., 1982; Bianchi et al., 2006; Holm and Fletcher, 1997; McKee and Sams, 1997, 1998; Sams, 1999). On the other hand, some researchers (Debut et al., 2003; Petracci et al., 2001; Sandercock et al., 2001) have reported no effect of acute heat stress on quality parameters of broiler breast meat. Holm and Fletcher (1997) and Sandercock et al. (2001) did not report any significant effect of acute heat stress on color of breast meat. Contrary to all other studies, Froning et al. (1978) reported darker (lower L*) and redder (higher a*) broiler breast meat as a result of heat stress. Higher shear values for breast meat of the acutely heat stressed (exposure of 1 h to 42ºC; 2 h to 40ºC; 4 h to 38ºC) birds were reported by Froning et al. (1978), Babji et al. (1982) and Holm and Fletcher (1997), respectively. Consistent with these reports, several other studies (Simpson and Goodwin, 1975; Lee et al., 1976; Petracci et al. 2001[only for 2 h boned fillets]) have also indicated that birds held in warmer temperatures yield tougher meat than that from birds held at cooler temperatures. But, Wood and Richards (1975) found no effect of heat stress (43ºC for 3 h) on breast meat tenderness. It should be noted that the influence of acute heat stress on meat quality could vary according to the condition of application (duration or intensity), which could explain some of the contrasting reports discussed above. A greater effect of environmental condition was reported on thigh meat color and pH compared to breast meat for both chickens (Kannan, 1997; Debut et al., 2003) and turkeys (Le Bihan-Duval et al., 2003). Debut et al. (2003) reported a decline in pH, color and curing cooking yield for the thigh meat as a result of acute heat stress, whereas Froning et al. (1978) did not find any effect of acute heat stress on color of thigh meat.

Cold environmental conditions prior to slaughter were reported to affect broiler and turkey breast meat by causing an increase in pH_u of the meat (Babji et al., 1982; Froning et al., 1978; Holm and Fletcher, 1997). Cool environmental conditions were studied by placing birds in a chamber at 5ºC, immersing birds into 4°C ice water for 20 min, or placing birds in environmental chambers set at 7 or 18ºC by Babji et al. (1982), Froning et al. (1978), and Holm and Fletcher (1997), respectively. In the most extreme cold condition birds were exposed to -20ºC for 6 h. In all of the aforementioned studies, cold stress seemed to produce better meat quality characteristics as there was a

moderate increase in pH_u of the meat that caused slightly darker color and higher water holding capacity. The increase in pH_u was related to the decrease in substrate availability for post-mortem metabolism since the initial pH and glycogen concentration were lower in the cold-stressed compared to the heat-stressed birds, but were not different from the controls (Lee et al., 1976). Reports on the effect of cold stress on tenderness of broiler chickens are contradictory, where Lee et al. (1976) did not find any significant effect of extreme cold stress on tenderness of broiler breast meat, but Babji et al. (1982) and Froning et al. (1978) reported more tender breast meat as an effect of moderate cold stress on broiler and turkey breast meat respectively.

Therefore, environmental conditions that birds are exposed to prior to slaughter could dramatically affect subsequent meat quality. The effect of heat stress prior to slaughter has been extensively studied, whereas studies on the effect of cold stress and extreme cold stress as observed in Canadian winters on broiler and turkey breast and thigh meat are very limited. More studies are required to establish the effect of cold stress on broiler post-mortem metabolism and breast and thigh meat quality.

2.2.3 Effect of transportation on bird physiology and meat quality

During transportation of broiler chickens from the farm to the slaughter plant, birds are exposed to a number of stressors resulting in reductions in both welfare and productivity. Known stressors include heat stress (due to high temperature and humidity), cold stress (due to wind at high vehicle speeds and wet feathers), crowding (inability to display thermoregulatory, social stress), vibration, acceleration, and noise (Mitchell et al., 1994; Savenije et al., 2002). Each of these factors and their various combinations may enforce stress on the birds, but thermal challenges and specifically heat stress constitute the major threat to animal welfare and productivity (Mitchell et al. 2001; Weeks and Nicol, 2000). The existence of thermal loads in transit could result in moderate to severe thermal stress and reduced welfare or increased mortality due to heat or cold stress (Hunter et al., 1999, 2001) and eventually affect product quality. However, the dead on arrival (DOA) might be associated with many factors including season, geographical location, journey length, size of bird, stocking density, health

status, vehicle design and slaughterhouse design and practice and it is very difficult to determine that which caused the death of the bird (Mitchell and Kettlewell, 2009).

Mitchell et al. (1994) demonstrated differential distributions of thermal conditions and stress responses depending on the location of birds within the vehicle, using ambient temperatures of 7.8 to 21.2ºC during transportation. Stress indicators included plasma enzyme activities and leucocytes counts. The thermal load within the vehicle due to temperature and humidity gradients coming from the metabolic heat and water production of the birds emphasizes the importance of the ventilation system during transportation. Specifically, in the summer when the curtains are open the load is relatively homogeneous in terms of temperature and humidity but reduced ventilation in the winter due to closure of the curtains leads to the existence of a thermal core in which the risk of heat stress is increased (Mitchell et al., 1994).

Transport of broilers in a well-ventilated vehicle could be done safely at ambient temperatures down to -4ºC in dry conditions (Hunter et al., 1999). However, under wet conditions, temperatures as high as 8ºC also induced moderate hypothermia. The combination of wetting and air movement increased evaporative and convective cooling to a degree that broiler chickens could not compensate by physiological regulation and metabolic thermogenesis, which caused a maximum and potentially lethal decrease of 14.2±5.47ºC in rectal temperature of birds at -4ºC. Rectal temperature dropped by 7ºC after 90 min of exposure to -4ºC under wet conditions, and by another 11.5ºC in the remaining 90 min. Withdrawal of food may also worsen the situation by leaving birds without a readily available source of metabolic energy for thermogenesis and thus accelerating their susceptibility to acute hypothermia. Therefore it is important to limit water entrance at ventilation inlets, yet maintain thermal homogeneity throughout the bio-load.

Transportation stress has been reported to affect the subsequent breast and thigh meat quality, however the reports are contradictory. Owens and Sams (2000) reported significantly higher muscle pH (at 0, 2, and 24 h), lower L* values (at 2 and 24h), higher marination retention and lower cook loss in the marinated fillets of transported turkeys compared to non transported turkeys. However, no significant difference in drip

loss and cook loss of the non marinated fillets or the marination uptake percentage between the transported and non transported turkeys breast meat were observed by Owens and Sams (2000). Bianchi et al. (2006) showed a reduction in broiler breast meat redness (lower a*) with an increase in transportation time and distance. Debut et al. (2003) observed significantly higher pH, darker color and higher curing cooking yield for thigh meat of transported birds (for 2 h of transport) but no effect on the breast meat quality of transported birds was observed. Breast meat was affected by physical activity of birds on the shackle line, with higher activity resulting in redder color and higher drip loss. Warriss et al. (1993) showed a reduction in *Bicep femoris* glycogen content with an increase in transport time, but not for the *Pectoralis* major muscle and related this difference between the muscles to the involvement of *Bicep femoris* or leg muscle in maintaining balance in the moving vehicle during transportation.

From all these studies, it can be concluded that transportation is a multi-component phenomenon, which greatly impacts bird physiology and could have a glycogenolytic effect on the muscle by imposing acute demands on energy metabolism, which in turn affects the pH_u and subsequent meat quality. The contradictory reported effects of transport on meat quality might be due to different environmental conditions during transport, length of transport and other factors such as gender, age, and lairage prior to slaughter, therefore effect of transport is not separate from these factors and cannot be considered alone. Extensive studies have been conducted on heat stress during transportation. However, studies on the effect of transportation under cold conditions are very limited and only available on some physiology aspects and not on the muscle metabolites and meat quality parameters; therefore it is necessary to assess the effect of cold transportation on energy reserves of broiler chicken breast and thigh muscles and meat quality.

2.2.4 Effect of genetics, gender and age of the birds at slaughter on meat quality

A number of studies have looked at the effect of genetic line and growth rate on quality of broiler breast meat (Anadon, 2002; Berri et al., 2001, 2005; Bianchi et al., 2006; Debut et al., 2003, 2005). Selection for growth rate and increases in body and

breast weight have been associated to different rate and extent of post-mortem pH decline (Berri et al., 2001), and different struggling activity on the shackle line (Berri et al., 2005; Debut et al., 2005). In all of these studies a fast growing line was associated with lower level of activity on the shackle and decrease in the rate and extent of pH drop. In addition, Berri et al. (2005) showed that struggling activity on the shackle line was strongly negatively correlated to *Pectoralis* major muscle pH at 15 min post-mortem and moderately negatively correlated to GP, which represents glycogen content at death in birds from different growth lines. In addition, Berri et al. (2007) reported that higher breast weight and yield was associated with larger fiber cross sectional area, higher pH at 15 min and 24 h post-mortem, darker color, lower drip loss and was better adapted for further processing. Bianchi et al. (2006) did not find any effect of the two commercial genotypes (Ross 508 and Cobb 500) on color coordinates (L*, a*, b*) of broiler breast meat.

Gender of an animal may influence post-mortem metabolism due to different responses of genders to pre-slaughter stress. Reports of the effect of gender on breast meat quality are contradictory, where Ngoka et al. (1982) reported no effects of gender on breast muscle pH, WHC, cooking loss, and color L*, a* and b* of turkeys. In a comprehensive study by Anadon (2002) on broiler chickens, it was reported that *Pectoralis* major muscle of female birds exhibited lower pH values at all times post-mortem, higher L*, a* and b* values, and lower WHC compared to males, which were significantly heavier than females (Anadon, 2002). Muscle of females showed a greater rate and extent of pH decline (pH_u = 5.69), lighter, redder and yellower breast meat color and lower WHC (15.8%) compared to males (pH_u = 5.84; WHC = 18.8%). These differences appeared to be the result of a higher degree of protein denaturation in muscles from female birds that exhibited lower pH values at all post-mortem time periods. The higher a* values for females reported by Anadon et al. (2002) were contradictory to other studies reporting a higher myoglobin concentration in the male muscles, and further, a pale color is usually associated with lower a* value.

Several studies have looked at the effect of broiler age on subsequent meat quality (Anadon, 2002; Bianchi et al., 2006, 2007; Sandercock et al., 2001; Smith et al.,

2002). Sandercock et al. (2001) reported lower pH immediately post-slaughter, lower shear values and higher pH_u and drip loss for 35 d old birds compared to 63 d old birds, which were speculated by the authors to be due to a greater degree of post-mortem glycolytic metabolism in the more mature muscle. Anadon (2002) reported higher WHC for breast fillets from 53 d old birds than 42 d old birds despite the lack of any significant differences in pH, L*, and R-values at 24 h post-mortem. However, pH and R-values at 0.25 and 4 h post-mortem were significantly higher in birds processed at 53 d, resulting in improved protein functionality and higher water holding properties of breast meat for these birds (Anadon, 2002). Results of the Anadon et al. (2002) study suggest that rigor mortis and post-mortem glycolysis occurred somewhat faster in younger than in older birds, which is contrary to Sandercock et al. (2001) findings. In a subsequent study from the same PhD. dissertation, Anadon (2002) reported an age related change in color of *Pectoralis* major muscle, where, L* values tended to increase linearly with increasing age at slaughter, which did not agree with their earlier study.

The reports of genetics, age and gender at slaughter failed to show the same effects on breast meat quality, which reflects the differences in bird selection, different ages that have been studied or different conditions applied in various studies. Therefore one could not draw a conclusive statement on the effect of these factors on meat quality, other than genetics, sex and age might play important roles in post-mortem metabolism and meat quality of broiler breast meat.

2.2.5 Effect of muscle fiber type on meat quality

Meat quality is strongly determined by the histological and biochemical characteristics of muscle fibers. Fiber type classification has been done based on numerous criteria, including, fiber color (red or white), contraction speed (fast and slow), and type of metabolism (primarily glycolytic or oxidative). The structure and function of muscle fiber types are revealed in metabolic differences between red and white fibers (Le Bihan-Duval, 2004). Muscles are classified as red or white based on proportion of white and red fibers they have. There is also an intermediate fiber type that has intermediate characteristics of both red and white fibers. Red fibers are smaller

in size, myoglobin and hemoglobin rich, have lower glycolytic potential, lower glycogen content, contain more lipid and are adapted to aerobic (oxidative) metabolism for slow, fatigue-resistant activity (Barbut, 2002; Dransfield and Sosnicki, 1999). In contrast white fibers are larger in diameter, adapted to anaerobic (glycolytic) metabolism, fast fatiguing, and used for brief length of activity (Barbut, 2002; Dransfield and Sosnicki, 1999). White fibers are well endowed with glycolytic enzymes that enable them to obtain energy rapidly by the incomplete oxidation of glycogen, which explains why white fibers soon become fatigued once their glycogen stores are depleted and lactate is accumulated. White fibers have higher glycolytic potential, higher amount of glycogen, lower oxidative metabolism and lower heme pigments compared to red fibers (Barbut, 2002; Pearson and Young, 1989; Le Bihan-Duval, 2004). White fibers are more susceptible to the PSE defect because they are highly dependent on glycolysis to preserve homoeostasis of muscle fibers after animal death, whereas red meat is less susceptible to the development of PSE because of the higher degree of red oxidative fibers in the muscles (Le Bihan-Duval, 2004).

Red fibers are shown to have greater ability to synthesize protein and therefore they contain more RNA and are supplied with more abundant blood flow compared to the white fibers (Barbut, 2002). On the other hand white fibers have better developed sarcoplasmic reticulum, most probably due to faster contraction and relaxation rates (Pearson and Young, 1989). The activity of all glycolytic enzymes including, both Ca^{2+} and Mg^{2+}-activated ATPase, glycogen synthase, PFK, lactate dyhdrogenase and fructose-1, 6-bisphosphatase activities of white fibers have been shown to be much higher than that of red fibers (Pearson and Young, 1989). Physiological types of muscle fibers behave differently once an animal has been slaughtered and its muscles are being converted to meat. In addition, diversity in work patterns of living muscles results in differences in myoglobin and lipid concentrations and connective tissue content, which have a major effect on meat quality properties (Swatland, 1994).

Classification of poultry meats are based on overall color of the meat, which is related to the relative proportion of red and white fibers within the muscle. In general most muscles are composed of a mixture of red and white fibers (Barbut, 2002), and

there are only few muscles with all white (*Pectoralis* major) or red fibers. The histological measurements of fiber type distribution of broiler breast and thigh muscle based on α-white (glycolytic), α-red (oxidative glycolytic), and β-red (oxidative) type fiber have classified breast and thigh meat to 98.2, 1.8, 0% and 75.6, 12.4, and 12.0% proportion of each fiber type respectively (Papinaho et al., 1996).

The pH_u is different for white and red fiber type muscles, where red leg muscle reaches pH_u of 5.9-6.0, within 2-3 h post-mortem, whereas white breast muscle pH may continue to decline beyond 24 h post-mortem to reach pH values of 5.4-5.6 (Lyon and Buhr, 1999). Higher pH_u values are reported for breast (5.7-6.0) (Barbut et al., 2002, 2005; Debut et al., 2003) and thigh (6.0 to 6.3) (Debut et al., 2003) muscles in other studies. However, pH_u for broiler breast fillets may also be attained by 8 h post-mortem (Khan, 1974) depending on energy reserve at time of slaughter.

Anaerobic muscles such as, *Pectoralis superficialis* (homogeneously white fiber) and *Posterior latissimus dorsi* (predominantly white fiber) were reported to have higher glycogen content compared to aerobic muscles such as *Sartorius* (predominantly red fiber) and *Anterior latissimus dorsi* (homogenously red fiber) (Sams and Janky, 1990). In addition, anaerobic muscles use glycogen as the primary energy storage compound, compared to lipid being used by aerobic fibers as the primary energy store (George and Berger, 1966; cited in Sams and Janky, 1990). Sams and Janky (1990) reported that glycogen stores were depleted within 3 h post-mortem in *Pectoralis superficialis* and within 2 h in *Posterior latissimus dorsi*, whereas, no significant change in glycogen level of the *Anterior latissimus* dorsi was observed post-mortem. Initial lactic acid concentrations were higher in anaerobic muscles compared to aerobic muscles (Sams and Janky, 1990). The R-value, ratio of IMP (inosine monophosphate) to ATP, for both red and white muscles increased after slaughter, where R-value of red muscle reached a plateau after 2 h, whereas that of white muscle continued to increase with time post-mortem until 8 h (the maximum time tested) (Sams and Janky, 1990). Higher R-values are indicative of higher proportion of IMP and therefore accelerated consumption of ATP due to rapid post-mortem glycolysis (Soares et al., 2007). In addition, the starting level of glycogen for post-mortem metabolism in different fiber

types depends on depletion and repletion patterns that occur during transport and ante mortem holding. The fibers responsible for shivering are mostly the slow-contracting type; therefore animals exposed to severe cold condition prior to slaughter may have less glycogen in these fibers (Lupandin and Poleshchuk, 1979; cited in Swatland, 1994). Therefore, these differences between red and white muscles in post-mortem metabolism cause the difference in quality parameters between the two muscles.

2.2.6 Conversion of muscle to meat

After slaughter, biochemical changes occur in the conversion of muscle to meat, which is determinant of final meat quality. Rigor mortis development is crucial in the process of muscle death and is essential to proper meat quality (Sams, 1999). Muscle cells stay alive after animal death and they continue to respire, producing and consuming energy in form of adenosine triphosphate (ATP). As cellular oxygen is depleted, the cells continue their activity using anaerobic metabolism to produce the needed ATP. Glycolysis is a fundamental biochemical process in the post-mortem conversion of living muscle to meat, which involves the breakdown of glycogen to glucose 6-phosphate (G6P) and glucose and then in the absence of oxygen to lactic acid in order to form ATP. The generated ATP from this reaction is utilized to continue muscle metabolism after animal death (Hartschuh et al., 2002). As there is no blood flow to remove the accumulated lactic acid out of the system, sarcoplasmic pH decreases to a level that slows down further glycolysis, and ATP production ceases. However, ATP consumption continues for a while (to detach actin and myosin). When the ATP concentration falls to a critical level (1 mmol/g), there is not sufficient ATP to separate all of the actin and myosin bridges, therefore these proteins remain as an actomyosin complex and muscle lose its extensibility. The actomyosin complexes continue to form until the ATP concentration reaches about 0.1 mmol/g, when rigor mortis is developed (Sams, 1999).

The conversion of muscle to meat is completed when muscles have used their energy reserve or have lost the ability to use the remaining reserve. For a short time after exanguination, ATP may be resynthesized from creatine phosphate (CP). After CP

has been used up, the length of time before the occurrence of rigor mortis depends on the amount of glycogen available within the muscle and continued activity of glycolytic enzymes (Swatland, 1994). The process of rigor mortis, which in latin means "stiffness of death" follows the depletion of energy from the muscle and results in a temporary toughening of the muscle. Because of the gradual depletion of glycogen and other energy sources (CP) within the cell, rigor mortis starts at a certain time after slaughter. This lag time is called the delay phase (Lyon and Buhr, 1999; Barbut, 2002). The length of the delay phase depends on pH_u and the elapsed time taken to reach ultimate pH (i.e. high pH_u reached rapidly corresponds to a short delay phase). However, the onset of rigor is not triggered at a specific pH, but occurs irrespective of pH when over 60% of the initial ATP is utilized (Khan, 1975). Onset of rigor in breast meat can occur within 15 min post-mortem, whereas it happens within 3 min in leg muscle. However, full rigor for breast fillets occurs at 2-4 h post-mortem compared to less than 2 h for leg meat (Kijowski et al., 1982). At this time muscle is inextensible, but after a certain period of time the muscle starts to become flexible again as a result of the breakage of sarcomere components due to the activity of proteolytic enzymes. Some of the major changes during the so-called aging process include the degradation of the z-line (leading to disintegration and weakening of the myofibrils) and degradation of the proteins titin, nebulin and desmin. The major proteolytic enzymes are calpains and cathepsins, which vary in their calcium requirements for activation. Calcium is available as it was released from the sarcoplasmic reticulum and mitochondria during post-mortem aging (Barbut, 2002).

Biochemical changes occurring in the conversion of muscle to meat can be affected at all stages of production, both pre and post slaughter and variations in the rate and extent of these changes in turn affect the sensory and functional properties of raw meat and further processed products (Dransfield and Sosnicki, 1999). However, the muscle energy reserve at slaughter and the enzymes involved in conversion of this energy reserve to lactic acid with concurrent energy production for post-mortem contraction play the most important part in the conversion of muscle to meat as discussed below.

2.2.6.1 Effect of energy reserve at slaughter on conversion of muscle to meat

The primary storage carbohydrate in muscle fibers is glycogen, which exists as single granules or clumps of granules located in the sarcoplasm between myofibrils and under the cell membrane. Glycogen is a polysaccharide formed by linking together of large numbers of D-glucose units polymerized by α 1-4 glycosidic bonds. Each chain has an average length of 13 glucose residues, and each internal chain has two branching points by 1-6 glycosidic bonds generating two new chains (Meléndez et al., 1997). A glycogen molecule has a protein in the core with branches of glucose linked together forming molecules with different molecular weight depending on the number of glucose chains. Creatine phosphate acts as a short term store of energy since it has a phosphate that can be quickly utilized in a chemical reaction to rephosphorylate ADP to ATP by the enzyme creatine kinase (CK) (Scheffler and Gerrard, 2007). CP is the dominant carrier of energy from the mitochondria to the myofibrils (Swatland, 1994). In addition, myokinase catalyzes the conversion of two adenosine diphosphates (ADP) to adenosine monophosphate (AMP) and ATP. However, creatine phosphate works best when oxygen is available, but if contraction proceeds rapidly and oxygen becomes limiting, the muscle will switch to anaerobic glycolysis to supply the energy needed for continuation of metabolism.

ATP production is necessary to keep muscle in a relaxed state post-mortem. This ATP is initially provided by creatine kinase and myokinase catalyzed reactions, but when 70% of the CP is degraded, ATP is replenished through glycogenolysis and glycolysis to prevent formation of permanent actomyosin crossbridges (Bendall, 1951; cited in Scheffler and Gerrard, 2007). Anaerobic glycolysis produces lactate and H^+, which cannot be removed from the muscle, causing a reduction in pH of the meat. The rate and extent of pH decline during the conversion of muscle to meat significantly impact the development of fresh meat quality characteristics (Scheffler and Gerrard, 2007).

The accumulation of lactic acid and resultant pH decline in meat are believed to be dependent mainly on glycogen present in the muscle at time of slaughter (Price and Schweigert, 1987). Therefore, glycogen concentration can be used as a means to

determine ultimate pH of the meat. Glycolytic potential (GP), which is a measure of lactate combined with potential lactate produced from glycogen, reflects the usual amount of steady state glycogen stored in muscle that could be converted to lactate, plus the lactate concentration at time of sampling. Therefore GP is less sensitive to sampling time and sample handling than either glycogen or lactate measured alone (Hartschuh et al., 2002). Several researchers have studied the relationship between muscle GP and meat quality of cattle (Immonen et al., 2000), pigs (Hambrecht et al., 2004b; Hambrecht et al., 2005) and poultry (Berri et al., 2001), respectively.

2.2.6.2 Glycogenolysis and glycolysis

Glycogenolysis is the enzymatic degradation of glycogen, and is the first step in the release of energy by the oxidation of glucose units (glycolysis). In glycogenolysis, the glycogen stored in the muscle is converted first to glucose 1-phosphate (G1P) and then into G6P. The two major enzymes involved in glycogenolysis are glycogen phosphorylase (GPhos) and glycogen debranching enzyme (GDE) (Yiä-Ajos et al., 2007). GPhos catalases the sequential separation of glucose units from the reducing ends of the glycogen molecule in the form of G1P until the fourth glucose before the branch point, and GDE eliminates the branch point and releases a free glucose and allows GPhos to continue to release G1P (Yiä-Ajos et al., 2007). Therefore, G1P and glucose are products of glycogenolysis in a ratio indicating the proportion between the mean length of straight chains and the number of branch points (Swatland, 1994). The activities of the two glycogen degrading enzymes were reported to be different between poultry white and red muscles, with higher activity of GPhos and lower activity of GDE in light *Pectoralis superficialis* (PS) compared to dark *Quadriceps femoris* muscles (Yiä-Ajos et al., 2007). GDE activity was reported to be over three times higher in *Quadriceps femoris* muscle compared to PS muscle. GDE activity was lower in both poultry muscles compared to porcine muscles (12 fold higher in *longissimus dorsi* muscle than PS muscle), whereas GPhos activity was higher in poultry muscles especially in PS compared to porcine muscles. Furthermore, the ratio between GPhos to GDE was higher in PS muscle than *Quadriceps femoris* muscle. However, a similar level of GPhos has been reported for muscles exhibiting fast and slow rate of glycolysis

in swine (Scheffler and Gerrard, 2007). On the other hand, GPhos exists in two forms: the less active, non phosphorylated form (GPhos *b*) and the more active, phoshorylated form (GPhos *a*), but researchers have not been able to establish differences in the activities or structural and kinetic characteristics of GPhos *a* and GPhos *b* from muscles of normal and PSE pork (Scheffler and Gerrard, 2007).

Enzymes catalyzing the reactions of glycolysis affect the rate and extent of pH decline by directly controlling the conversion of metabolites through the pathway (Figure 2.1). Phosphoglucomutase catalyzes the isomerisation of G1P to G6P, which can then proceed through glycolysis. Fructose 6-phosphate is produced from G6P by phosphoglucose isomerase, which is then converted to fructose 1, 6-bisphosphate by the enzyme phosphofructokinase-1 (PFK-1). Pyruvate kinase catalyzes the irreversible conversion of phosphoenolpyruvate and ADP to pyruvate and ATP. A 10 fold increase in the activity of pyruvate kinase was reported for PSE meat of the halothane susceptible pigs compared to the normal meat, which was related to a shift in pyruvate kinase activity due to phosphorylation of this enzyme, resulting in an additional more acid stable isoform. However, this difference in activity was not observed in PSE muscle from pigs free of the halothane gene (Scheffler and Gerrard, 2007). In the review by Scheffler and Gerrard (2007) it was concluded that properties of the rate limiting enzymes did not seem to be different between the normal and PSE muscles and therefore other glycolytic regulators could contribute to altered glycolysis by manipulating enzyme activity based on energy demand of the cell.

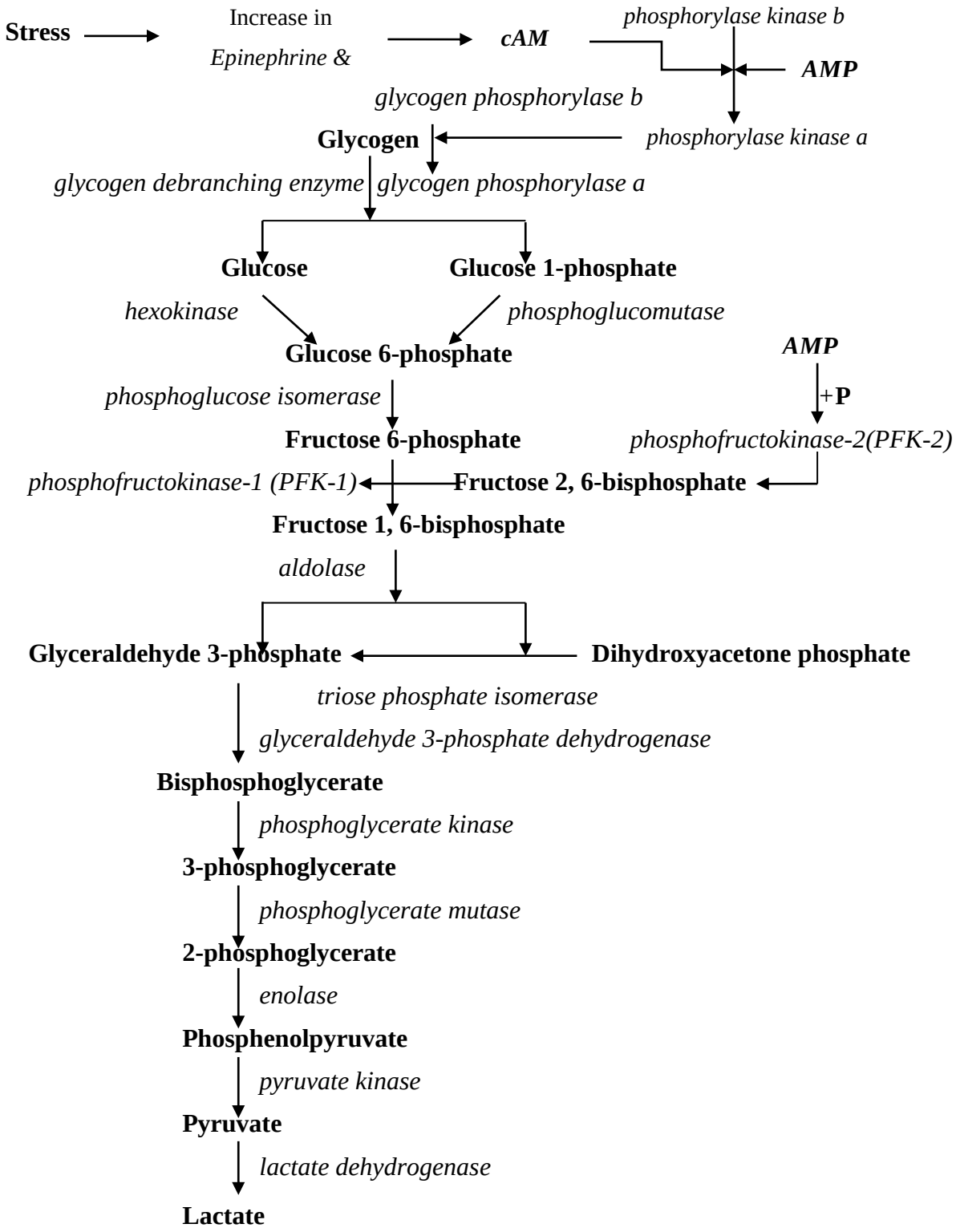

Figure 2-1 Enzymes and metabolic intermediates of the glycolytic pathway (modified from Scheffler and Gerrard, 2007).

In muscle of a live animal, the regulation of glycolysis is integrated with the metabolic state of the fiber and its immediate energy needs. The metabolic state of the fiber is affected by hormones, particularly epinephrine, and extent of the recent activity of the fiber. GPhos is predominantly important in the conversion of muscle to meat (Figure 2.1)because it is a primary control site for post-mortem glycolysis (Kastenschmidt et al., 1968; cited in Swatland, 1994). GPhos activity is regulated by phosphorylation and allosteric mechanisms. GPhos is most active when it is phosphorylated (GPhos *a*), and less active when it is dephosphorylated (GPhos *b*). Phosphorylase kinase (PK) exists in two forms, PK *a* and PK *b*, which are both capable of converting GPhos *b* to active GPhos *a* form. PK is most active when PK *b* is phosphorylated to PK *a* and binds to Ca^{2+}. PK *b* requires higher concentration of Ca^{2+} for activity; however, the Ca^{2+} concentration in the sarcoplasm during contraction is sufficient to activate PK *b* in order to convert GPhos *b* to GPhos *a* (Connett and Sahlin, 1996).

In addition, secretion of the hormones epinephrine and glucagon induce cAMP formation that leads to phosphorylation of PK *b* to PK *a* (Figure 2.1). In stressful conditions, epinephrine mediated activation of PK *b* and subsequent activation of GPhos *b*, which results in increased glycogenolysis. The conversion of GPhos *b* to GPhos *a* is inhibited locally in resting muscle by high concentrations of ATP, ADP and G6P. However, the effect of these substances may be overcome by increase in AMP and IMP that leads to GPhos *b* activation. Furthermore, the sensitivity of GPhos *b* for each factor is dependent on the concentration of substrate (glycogen and inorganic phosphate) and product (G1P) (Scheffler and Gerrard, 2007). If the produced energy is not consumed rapidly, the energy release mechanism will shut down. On the other hand if the energy is used, AMP and phosphate (from ATP→ADP+P; ADP→AMP+P) will further enhance the activation of GPhos (Swatland, 1994).

Research has shown that in muscles with a normal rate of glycolysis, G6P levels tend to decrease during the first h post-mortem and increase thereafter, suggesting that GPhos may not be able to supply adequate G6P to keep up with glycolytic metabolism, resulting in a rapid utilization of the G6P pool. This was speculated by authors in the

review of Scheffler and Gerrard (2007) that high levels of ATP and low levels of AMP and IMP following exanguination were insufficient to activate GPhos *b*, but due to the changes happening during the first h post-mortem in the relative concentrations of the allosteric activators, GPhos *b* might be activated. Therefore increase in GPhos activity, or decrease in PFK activity or other enzymes downstream might be responsible for the increase in G6P after one h post-mortem (Scheffler and Gerrard, 2007). PFK activity depends on the energy status of the muscle cell and it is stimulated as the ATP/AMP ratio decreases. In addition, hexose bisphosphate and inorganic phosphate could also activate PFK. But, ATP is required for the transfer of a phosphate group to fructose 6-phosphate. In fast glycolyzing muscles, rapid depletion of ATP could compromise the ability of PFK to catalyze the formation of fructose 1, 6-bisphosphate. On the other hand in slow glycolyzing muscles the imbalance between fructose 6-phosphate and fructose 1, 6 bisphosphate after 60 min post-mortem suggests a partial inactivation of PFK due to decrease in pH. Therefore the relative levels of glycolytic regulators and rate limiting enzymes contribute to altered post-mortem glycolysis (Scheffler and Gerrard, 2007).

One of the key enzymes in post-mortem glycolysis is AMP-activated protein kinase (AMPK) being a heterotrimer comprising of one catalytic subunit (α) and two regulatory subunits (β and γ) (Carling, 2004). *In vivo* studies have shown that AMPK is activated in ischemic cardiac muscle and hypoxic skeletal muscle and it is an interesting enzyme to be investigated due to its role in enhancing post-mortem metabolism (Kim et al., 2004). AMPK is activated following ATP depletion or, more accurately, a rise in the AMP/ATP ratio within the cells. When muscle is low in oxygen, it switches to anaerobic glycolysis, thereby the AMP: ATP ratio increases, which will allow AMP to bind to AMPK making it a better substrate for phosphorylation by its upstream kinase, AMPKK (Corton et al., 1994). The crucial role of AMPK in maintaining energy balance within cells is due to its sensitivity to the intracellular ATP level during post-mortem metabolism (Carling, 2004; Hardie, 2004). AMPK regulates glycolysis through two major pathways (Figure 2.1). First it activates phosphorylase kinase, which then activates GPhos and promotes glycogenolysis (Russell et al., 1999), and secondly, it

phosphorylates phosphofructo kinase-2, which catalyzes the formation of fructose 2, 6-bisphosphate that is an allosteric activator of phosphofructo kinase-1, a key rate-limiting enzyme of glycolysis (Marsin et al., 2000). Thus, AMPK activation indirectly increases glycolysis making it a strong candidate for modulating meat quality development. Research has shown that if the effect of AMPK is eliminated in post-mortem metabolism, glycolysis and pH decline is inhibited (Shen et al., 2007).

The role of AMPK activity in controlling post-mortem glycolysis was previously reported for the muscle of mice (Du et al., 2005; Shen and Du, 2005a, b; Shen et al., 2005) and pigs (Shen et al., 2006). Shen and Du (2005a) reported that AMPK was activated in post-mortem muscle at an early stage, where its activity increased 3 times within the first h after slaughter showing that it promotes post-mortem glycolysis. According to Shen and Du (2005a) low activity of AMPK is correlated with higher pH_u due to a decrease in glycolytic rate at the glycogenolysis stage, which prevents the drop in pH of the meat. In other studies by Shen and Du (2005b) and Du et al. (2005), a strong correlation between AMPK activation and pH decline in post-mortem mouse *longissimus dorsi* muscle was reported, further indicating that AMPK regulates glycolysis in post-mortem muscle. Shen et al. (2006) showed that AMPK reaches its maximum activity quicker in pigs that undergo transportation stress compared to the control, or those rested after transport. Consistent with these findings, (AMP+IMP): ATP ratio was greater in the transported pigs. Furthermore, PSE pork has been associated with early post-mortem activation of AMPK and higher activity of this enzyme immediately following slaughter (Shen et al., 2006). All these studies demonstrate the important role of AMPK in post-mortem metabolism of muscle.

The only study that has looked at AMPK activity in poultry, is the study by Sibut et al. (2008). These authors reported three times higher activity for AMPK in the breast muscle of lean broilers compared to the fat birds within the two experimental lines of chicken divergently selected on abdominal fatness (Sibut et al., 2008). The higher activity of AMPK in the breast muscle of lean birds was associated with darker breast meat with lower drip loss compared to the fat line. Furthermore, a greater gene expression was observed for glycogen synthase, GPhos, and the γ subunit of PK in lean birds. Findings of the Sibut et al. (2008) study are in contrast with the studies on the

activity of AMPK in porcine and mice models, where higher activity of AMPK was associated with lower pH and paler color. Therefore, AMPK activity might be different in broilers compared to other species, yet this needs further investigation.

2.2.7 Post-mortem temperature

Temperature of the carcass immediately after slaughter is an important factor influencing rate and extent of post-mortem metabolism and rigor mortis development and consequently overall meat quality characteristics. Temperature reduction post slaughter normally has a slowing effect on biochemical reactions due to slower rate of ATP depletion and thus a slower rate of rigor mortis development (Hamm, 1982; cited in Sams and Janky, 1990). Fast reduction in pH, while carcass temperature is high could cause toughening due to inactivation of calpain system, which plays an important role in post-mortem tenderization (Dransfield, 1994). Increase in myosin denaturation was reported as a result of faster pH decline (Offer, 1991) and elevated temperatures (Dransfield and Sosnicki, 1999) that could increase the likelihood of reduced water holding capacity and pale color similar to pale, soft and exudative (PSE) condition in pork (Offer, 1991), which will be discussed in the following section. Therefore, rate of carcass cooling could significantly affect subsequent meat quality. Elevated post-mortem temperatures (20 and 40ºC) are reported to accelerate the rate of post-mortem metabolism, ATP depletion and glycogen depletion in turkey *Pectoralis* muscles, which resulted in muscle with PSE characteristics (McKee and Sams, 1998; Owens and Sams, 1997). In addition, post-mortem holding temperatures of 40ºC are reported to result in higher drip and cook loss (McKee and Sams, 1998), shortened sarcomere length and higher shear values compared to temperatures of 0 and 20ºC for turkey breast meat (McKee and Sams, 1998). Furthermore, a delay in chilling post slaughter was shown to increase breast meat color lightness and decrease protein extractability and cook yield of the turkey breast meat compared to breast meat from the immediately chilled carcasses (Rathgeber et al., 1999). Different muscles respond differently to the rate of chilling, i.e. rate of pH decline was not affected by rapid chilling of *Longissimus lumborum* (LL) of pork, whereas a reduced rate of pH decline was observed for

Semimembranosus (SM) (Hambrecht et al., 2004a). In addition, Sams and Janky (1990) reported that development of rigor mortis is slowed by chilling only in red fibers, but not in white anaerobic fibers. Therefore proper chilling regimens are extremely important, because increased temperatures during post-mortem metabolism results in lighter color and tougher meat with increased drip loss and cook loss similar to the PSE meat condition.

2.3 Meat Quality Defects

2.3.1 Pale, soft, exudative defect

Pale, soft and exudative (PSE) meat is a quality defect in the meat industry, which accounts for huge losses particularly in the pork and poultry sectors. It was first described by Ludvigsen for pork in 1953 (Briskey, 1964). PSE meat development was earlier related to increased rate of early post-mortem glycolysis, indicated by elevated muscle temperature and rapid pH decline, which results in sarcoplasmic and myofibrillar protein denaturation and meat with pale color, soft texture and high water loss (Briskey, 1964). More recently, it's been shown that extended glycolysis and low ultimate pH could also result in PSE meat (Sellier and Monin, 1994). PSE pork occurs in 5-20% of pig carcasses, with reported 15.5% PSE in pork produced in the USA (Stetzer and McKeith, 2003). The tendency to produce PSE pork might have a genetic basis (porcine stress syndrome; PSS) (Eikelenboom and Minkema, 1974) or might be a result of excess glycogen content at time of slaughter (Monin and Sellier, 1985). Pigs with a single point mutation, Rendement Napole (RN), that was first found in the Hampshire breed (LeRoy et al., 2000) are very prone to develop PSE meat. PSE pork is undesirable and costly for both fresh and further processed products, by having 10-12% more drip than normal pork after 2-5 d of storage (Honikel, 2002), and showing as high as 40% cook loss (Briskey, 1964). PSE meat costs the pork industry around $30 million each year as reported by USDA (Smith and Northcutt, 2009).

More recently the PSE defect was also reported in turkey (Barbut, 1997, 1998) and chicken (Van Laack et al., 2000; Woelfel et al., 2002), respectively. PSE incidences

of 5-40% and 0-47% were reported respectively for commercial flocks of turkeys (Barbut, 1998; Owens et al, 2000) and chickens (Barbut, 1997; Woelfel et al., 2002). While the origin for development of PSE meat is already established in pork, details on characteristics and causes of PSE in broiler breast meat remain unclear. However, growing evidence suggests that it is very likely that thermal stress conditions immediately prior to slaughter is one of the causes of PSE meat in poultry (Barbut, 1998, McKee and Sams, 1998; Owens et al., 2000; Sams, 1999). Several authors have studied poultry stress syndrome (PtSS) to evaluate if PSE development in poultry is related to a stress syndrome similar to PSS related to PSE pork. Cavitt et al. (2004) did not find any relation between broilers sensitive to halothane gas (Hal^+) and development of PSE characteristics in breast meat. Owens et al. (2000) reported that PtSS could lead to PSE meat; however, the 3.5% turkeys identified as Hal^+ did not end up with a significantly higher incidence of PSE at slaughter. On the other hand, Soares et al. (2007) reported 47% incidence of PSE breast meat from the Hal^+ population. Therefore, the genetic basis of PSE development in broilers is not established as strongly as it is known in pork. However, Hal^+ broilers are prone to produce PSE breast meat, but PSE development in broilers needs further investigation. It is assumed that PSE in broiler breast meat is comparable to PSE pork meat (Barbut, 1998), but it is not established if PSE breast meat happens to the same extent as PSE pork meat. PSE turkey has pale color and poor processing characteristics (Pietrzak et al., 1997) similar to PSE pork, but the fresh meat drip loss was not comparable (Dransfield and Sosnicki, 1999). In addition, a number of studies have reported broiler breast meat to exhibit PSE symptoms similar to pork PSE (Barbut, 1997; VanLaack et al., 2000; Zhang and Barbut, 2005). However, in a review by Smith and Northcutt (2009) it was concluded that broiler breast meat selected as PSE are mainly based on the pale color, and might not truly be exhibiting PSE properties as seen for pigs. Furthermore, these authors mentioned that it might not be appropriate to use the term PSE for pale broiler breast meat, since it does not meet the criteria for PSE as originally derived from the pork industry due to very pale color, soft texture and excess drip. Currently, researchers are trying to better explain the PSE-like condition in poultry and find the possible origin or causes of this quality defect in the poultry meat industry, and some work has already

been published on the causes behind the PSE-like condition in poultry (McKee and Sams, 1997, 1998; Owens et al., 2000; Smith and Northcutt, 2009; Soares et al., 2007; Woelfel et al., 2000).

Pre-slaughter stress and struggling have been reported to accelerate metabolism in chicken and turkey breast meat, resulting in accumulation of lactic acid in the muscle right after slaughter to a level comparable to or even greater than that reported for PSE pork muscle (Ma and Addis, 1973; cited in Smith and Northcutt, 2009). A severe depletion in glycogen stores and increase in the level of lactic acid early post-mortem was reported for PSE breast meat (Berri et al., 2001). Slow chilling of turkey meat after slaughter was reported as an important factor in development of PSE-like meat characteristics (McKee and Sams, 1998; Rathgeber et al, 1999; Alvarado and Sams, 2004). From a morphological point of view, PSE meat is shown to have gaps of variable width between fiber bundles but no structural irregularities (Barbut et al., 2005). These intracellular open spaces between the muscle bundles, along with more extensive protein denaturation for PSE meat compared to DFD meat resulted in higher drip loss and unbound brine in the raw state of fresh and further processed products, respectively (Barbut et al., 2005).

Several studies have shown that early post-mortem pH might be an effective method of detecting low quality (PSE-like) turkey (Froning et al., 1978; Ngoka et al., 1982) breast meat. Turkey carcasses with low initial pH (< 5.7; fast glycolyzing) are reported to have higher drip loss and L* value and lower protein extractability compared to the medium pH (5.7 to 6.18) and high pH (> 6.18; slow glycolyzing) carcasses (Wynveen et al., 1999). In addition, cooked meat from the fast glycolyzing group was tougher than the normal glycolyzing group (Wynveen et al., 1999; Rathgeber et al., 1999; Molette et al., 2005). Consistent with these studies, Sandercock et al. (2001) also found that higher drip loss in the muscle of heat-stressed broilers was closely associated with the rate of muscle pH immediately post-slaughter. However, Owens et al. (2000) did not show the same relationship between pH at either 1.5 or 24 h post-mortem and drip loss in turkey meat, and reported that drip loss was significantly affected by L* value rather than the pH.

Different cut off points (truncation values) have been suggested by various researchers to classify poultry breast meat as PSE. McCurdy et al. (1996) suggested a cut off value of L* >50 for PSE turkey that was based on lower WHC above this range, whereas, Owens et al. (2000) proposed a cut-off point of L*> 53 for PSE turkey meat based on the relationship between pH, color and expressible moisture. Woelfel et al. (2002) on the other hand established a cut-off point of L*=54 based on poor WHC of meat with lightness of 54 and above. Barbut (1997, 1998) recommended a truncation value of L*> 49/50 and L*> 52/53 for broiler chickens and turkey hens respectively, whereas, Petracci et al. (2004) suggested a truncation value of L*=56 to identify paler than normal (PSE-like) broiler breast meat. However, it was recommended that each processing plant needs to determine its own lightness values for sorting PSE meat depending on type of birds, processing factors, and final product specifications (Barbut, 1997; Woelfel et al., 2002; Petracci et al., 2004).

From a consumer point of view, sensory aspects and nutritional values are the most important factors for quality evaluation. Komiyama et al. (2008) did not find any difference based on consumer liking of general appearance, flavor, and tenderness of cooked pale chicken breast meat and normal breast meat. Zhuang and Savage (2010) also did not find any effect of raw fillet color on flavor intensity of the cooked fillet. However, it was shown that the average intensity scores for mechanical texture attributes, cohesiveness, hardness, rate of breakdown, and chewiness of the light fillets were significantly higher than either the dark or the medium fillets, which were not different from each other (Zhuang and Savage, 2010). Therefore, PSE meat with a low pH can also affect sensory mechanical texture characteristics of cooked poultry fillets.

2.3.2 Dark, firm, dry defect

Meat with DFD condition is dark in color, has a firm texture and dry appearance, which are mainly related to its high pH and higher protein functionality resulting in higher water holding ability and in return a firm texture and dry surface characteristic for this meat (Barbut et al., 2005; Owens and Sams, 2000). The high WHC of DFD meat might increase its susceptibility to microbial contamination and therefore result in

a shorter shelf life for this meat (Allen et al., 1998). In addition, higher WHC of DFD meat results in lower light scattering from the surface and therefore a substantially darker color for DFD meat (Barbut et al., 2005; Swatland, 1994). Breast meat with DFD characteristics was investigated for both broiler chickens (Barbut, 1997, 2005; Qiao et al., 2001; Woelfel et al., 2002) and turkeys (Zhang and Barbut, 2005). From a morphological point of view the muscle fibers in DFD meat are arranged in a much denser and more compact manner compared to normal meat, which had a fairly loose microstructure with no abnormalities (Barbut et al., 2005). The cut off point for pH_u of poultry breast meat with DFD defect has been established at 6.1 or higher by the majority of researchers (Barbut, 1997, 1998; Barbut et al., 2005; Berri et al., 2001; Qiao et al., 2001; Petracci et al., 2004; Woelfel et al., 2002). However the cut off point for color lightness (L*) used for DFD classification has been set at different points among the aforementioned studies. Petracci et al. (2004) suggested $L^* < 50$, whereas, $L^* < 46$ has been recommended by Barbut (1997), Woelfel et al. (2002), Qiao et al., 2001; and Barbut et al. (2005). However, as previously mentioned it is suggested by Petracci et al. (2004) that cut off points should be determined for each processing plant based on flock and final product specifications.

It is believed that DFD meat is related to long-term stress before slaughter that causes depletion in muscle glycogen resulting in higher post-mortem muscle pH because of the prevention of glycolysis by elimination of its substrate (Owens and Sams, 2000). Many factors including transport exhaustion (Lesiow et al., 2007; Warris et al., 1999), feed withdrawal (Kotula and Wang, 1994), climatic stress, in particular cold stress (Nicol and Saville-Weeks, 1993; Webster et al., 1993), resting prior to slaughter (lairage time) (Warris et al., 1999), and aggressive behavior could contribute to the depletion of muscle glycogen and in return limit the amount of lactate formed post-mortem. The incidence of DFD breast meat in poultry has been studied by Lesiow et al. (2007) and Petracci et al. (2004). According to Petracci et al. (2004), breast meat color was significantly darker in winter compared to summer. Lesiow et al. (2007) further confirmed that transportation during winter season caused a significant increase in the incidence of DFD broiler breast meat. In a recent study by Zhuang and Savage (2010), no difference in average flavor intensity scores of breast meat was reported

between different groups categorized based on raw meat color (light, L*>60; medium, 55<L*<59; dark, L*< 55). In addition these authors did not find any difference in texture profiles between the cooked medium and dark fillets.

The characteristics of DFD breast meat are already established, however, the causes for this defect in the poultry industry are not clear. It has been shown that cold winters could increase the incidence of DFD breast meat in broiler chickens. However, more studies are required to establish the exact causes of this defect and the biochemistry behind DFD development in poultry breast meat. It is important to understand the challenges to lower the incidence of DFD in the poultry industry especially in the winter season and find solutions to use this meat properly in further processed products, i.e. by manipulating the pH.

2.4 Summary

Both visual and functional properties of meat contribute to product quality. Color, odor, flavor, juiciness and tenderness are among the most noticeable features of meat, determining consumer acceptability of the product. Quantifiable properties such as pH, water holding capacity, cook loss and tenderness are important factors that influence quality of fresh and further processed products.

All the steps from the 12-24 h period prior to slaughter, slaughtering procedure and post slaughter conditions influence meat quality parameters by affecting post-mortem metabolism. Heat and cold stress, loading and unloading, transportation, and handling contribute to pre-slaughter stress and can alter color, texture, and related protein functionality. Transportation is an essential component of the poultry industry but places an enormous stress on birds. Exposure of birds to extreme conditions of cold and wetting for short periods of transport might cause hypothermia in the birds, where the core body temperature drops to a degree that birds cannot physiologically regulate their metabolic thermogenesis. Withdrawal of food and water prior to transportation may worsen the condition by increasing bird susceptibility to acute hypothermia. In contrast, birds exposed to extreme conditions of heat for a short period of time will be hyperthermia, when the core body temperature increases beyond the critical limit, and

heat production cannot be reduced effectively. While the effect of heat stress has been extensively studied in poultry, there is no information regarding cold stress on the quality of the subsequent meat. In addition to pre-slaughter stressors, many factors, including genetics, age, gender and post-slaughter handling may contribute to variations in meat quality.

Post-mortem rate and extent of chemical changes in muscle is determinant of pH_u and subsequent quality of the meat. Many enzymes involved in glycogenolysis (the first step in using muscle energy reserves post-mortem) and glycolysis (process of converting glucose and G6P to lactic acid and producing ATP as energy) play important roles in post-mortem metabolism, among which AMPK may be a key enzyme in controlling rate and extent of post-mortem metabolism and subsequent meat quality. The important role of AMPK in post-mortem metabolism of mouse and pigs is already established, but its role in post-mortem metabolism of broiler chickens is still unknown.

Two conditions of PSE and DFD meat can develop in poultry meat as a result of ante-mortem stressors and their effect on post-mortem metabolism. PSE fillets have significantly lower pH, greater L* value, and lower WHC, which makes this meat less favourable for further processing products and less acceptable to consumers. Rapid post-mortem glycolysis and extended glycolysis are the main causes of PSE meat, which lead to rapid pH drop while carcass temperature is still high or low ultimate pH in meat. Factors including pre-slaughter heat stress could contribute substantially to the development of PSE defect in poultry. DFD fillets on the other hand have higher pH, lower L*, and higher WHC, making it a great choice for further processing due to its higher cook yield. However, the high WHC increases the susceptibility of DFD meat to microbial growth and shortens the shelf life; in addition dark color of the fresh skinless breast meat is not appealing to the consumers. The DFD defect was related to long term stress prior to slaughter, including transport exhaustion, handling, feed withdrawal and cold exposure, causing depletion in muscle glycogen content that in return decreases the potential lactate production, leading to a high pH_u. However, further studies are required to better understand the biochemical basis of DFD defect in broiler breast meat and estimate the incidence of this defect in the poultry industry. Effect of extreme cold

exposure prior to slaughter on meat quality attributes of chicken is unclear. In the following research project, bird physiology and meat quality parameters will be examined following extreme cold exposure during actual and simulated transportation. Furthermore, the biochemical basis of DFD defect in poultry breast and thigh meat will be explored.

3. EFFECT OF MICROCLIMATE TEMPERATURE DURING TRANSPORTATION OF BROILER CHICKENS ON QUALITY OF THE *PECTORALIS* MAJOR MUSCLE

3.1 Abstract

This study investigated the effect of microclimate temperature during pre-slaughter transportation on chicken meat quality. Ninety broilers per load of 2900 were monitored individually during 3-4 h of pre-slaughter transport in an actively ventilated trailer. Six transport test runs were conducted at average ambient temperatures of -27, -22, -17, -5, +4, and +11ºC. Birds were classified into four groups based upon the temperatures recorded in their immediate surroundings, as follows: -16–0, 0–10, 10–20, and 20–30ºC. Internal body temperatures of the birds were recorded using Thermocron DS1922L iButtons. Birds were slaughtered in a commercial facility and meat quality of the chilled carcasses was evaluated by determination of pH, color, drip loss, thaw loss, cook loss, shear force, water binding capacity (WBC), and pellet cook yield (PCY) of the *Pectoralis* major muscle. The breast meat from birds exposed to temperatures below 0ºC showed a significantly higher ($P < 0.05$) ultimate pH. Breast meat from birds exposed to temperatures below 0ºC showed significantly higher ($P < 0.05$) pH_u, a* value, WBC and PCY, and a significantly lower L* compared to breast meat of birds exposed to temperatures above 0ºC. The average core body temperatures were significantly lower ($P < 0.05$) during transport for birds exposed to temperatures below 0ºC compared to those exposed to temperatures between 0 and 10ºC. The latter birds had significantly lower ($P < 0.05$) core body temperature compared to those exposed to temperatures above 10ºC. Thaw loss was significantly higher ($P < 0.05$) for breast meat of birds exposed to temperatures above 20ºC during transportation. There was no

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significant trend for b* value, drip loss, cook loss or shear values based on environment temperature immediately surrounding the birds. Exposure to temperatures below 0ºC increased the incidence of dark, firm, dry (DFD) breast meat and decreased the incidence of pale, soft, exudative (PSE) breast meat. These results demonstrate that pre-slaughter transport may influence breast meat quality characteristics of broiler chickens.

3.2 Introduction

Transportation of broiler chickens is a stressful process, but it is an essential component of the poultry processing industry. Pre-slaughter transport and handling could increase stress on the birds, by decreasing muscle glycogen reserves and therefore affecting the rate and extent of pH drop which could affect the resultant meat quality (Owens and Sams, 2000; Debut et al., 2003). It is reported that pre-slaughter temperature affects the post-mortem metabolism of muscle via adrenal or other physiological responses or simply by fatigue of the birds (Petracci et al., 2001). Pre-slaughter heat stress has been reported to accelerate the rate and extent of rigor mortis development, post-mortem glycolysis, and post-mortem metabolism and biochemical changes in the muscle, resulting in undesirable changes in meat characteristics similar to the pale, soft, and exudative (PSE) condition (McKee and Sams, 1997; Sams, 1999; Sandercock et al., 2001). Exposure of chickens to heat stress prior to slaughter results in breast meat with lower ultimate pH (Holm and Fletcher, 1997; Sandercock et al., 1999), reduced water binding capacity (Sandercock et al., 1999; Petracci et al., 2001), and reduced tenderness (Froning et al., 1978; Holm and Fletcher, 1997; Petracci et al., 2001).

On the other hand a cold environment prior to slaughter might also cause stress to the bird and potentially affect meat quality. Several authors have reported that exposure of chickens to cool temperature conditions (4, 5 and 7ºC) prior to slaughter results in breast meat with better functional properties, due to higher pH (Froning et al., 1978; Babji et al., 1982; Holm and Fletcher, 1997). However, the effect of exposing chickens to sub-zero temperatures on breast meat quality has not been studied, even

though this is a special challenge for transporting chickens in winter particularly in Canada.

The objective of the present study was to investigate the effect of microclimate temperature during transportation on broiler breast meat quality; and the incidence of PSE and dark, firm and dry (DFD) breast meat under sub-zero transport conditions, using an actively ventilated transport vehicle.

3.3 Materials and Methods

This study was conducted at six different ambient temperatures of -27, -22, -17, -5, +4, and +11ºC. Ninety broiler chickens were placed in a set location inside the transport vehicle with the capacity of approximately 2900 birds as shown in Figure 3.1. However, this subset is not representative of the entire trailer, but did provide a range of environmental conditions for each test run performed. Birds were between 39 and 42 d of age and between 2.19 and 2.30 kg live weight. The birds were wing banded at the farm and temperature logging Thermocron iButtons (DS1922L iButtonÂ®, Maxim Integrated Products, CA) were inserted into the proventriculus of test birds to monitor their internal body temperature. This project was approved by the University Committee on Animal Care and Supply of the University of Saskatchewan, which is subject to the Canadian Council on Animal Care. Birds were then placed into a grid system (112cm x 71cm) within 6 drawers (15 birds/drawer) of a module-based handling system that is a component of the Anglia Autoflow modular system (IP22 1SR, Wortham Ling, Norfolk, England) (Figure 3.1). Each test bird was allocated a specified space with a Hygrocon iButton (Maxim Integrated Products, Sunnyvale, CA) data logger recording temperature and relative humidity (RH) during transportation. Up to 150 Hygrocon iButtons were strategically placed throughout the trailer for each test-run. The modules were transported, using an actively ventilated transport vehicle with 2 tarped sides and an insulated floor and ceiling. Ambient air temperature was recorded with a logger on the outside of the truck. Transport duration was 3-4 h for all the test

runs. The average body temperature during transportation was calculated based on data collected every minute.

After a short lairage, which varied between 30 min and 120 min depending on the arrival time to the slaughter plant (Lilydale Corp., Wynyard, SK.) birds were commercially slaughtered, using electrical stunning followed by exanguination of the carotid arteries and jugular veins. Birds were mechanically eviscerated after slaughter and removal of their feathers. Following evisceration, birds were taken off the line and were tagged, weighed and placed into the plant chilling system (0ºC, 30 min). Chilled carcasses were weighed once again prior to packing in boxes with ice and then sent to the meat laboratory of the University of Saskatchewan for quality assessment.

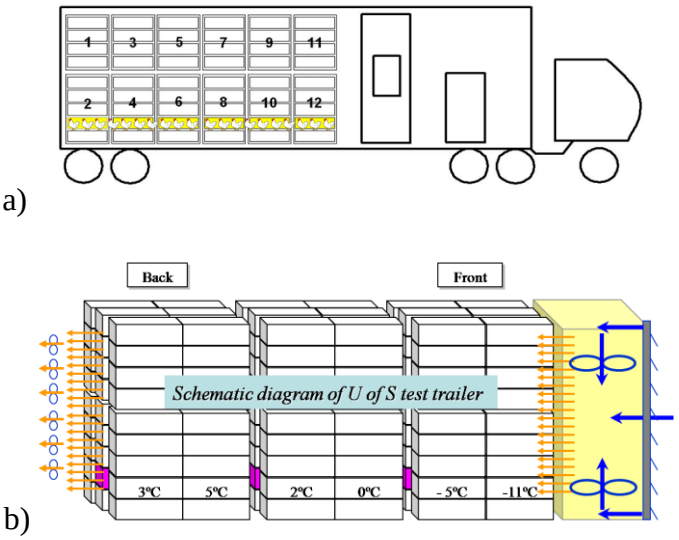

Figure 3-1 Schematic diagram of the University of Saskatchewan test trailer with 12 modules each holding 12 drawers (the number of the module is shown in the middle of each compartment), and birds being placed on the second plane from the bottom (a) and schematic diagram of modules inside the trailer (b), with average temperature of each drawer holding the test birds listed when the environment temperature during transportation is at -27ºC

3.3.1 Meat quality measurements

The carcasses were weighed, and manually deboned at 6-8 h post-mortem. The left and right breast meat pieces (*Pectoralis* major) were removed for evaluation of the quality parameters as listed below.

3.3.1.1 Drip loss

Breast meat samples were weighed and placed on soaker pads on Styrofoam trays, covered with plastic bags and stored at 4ºC for further testing. After 24 h refrigeration (approximately 30 h post-mortem) breast samples were reweighed and drip loss was determined as the difference between initial weight and the weight after 24 h drip (Wang, 2005). Drip loss is presented as percentage of weight lost during 24 h of refrigerated storage.

3.3.1.2 Color measurement

Meat color was evaluated at 30 h post-mortem (following the end of the 24 h drip test) using a Minolta Chroma meter (RC-400). The CIE system color profile of lightness (L*), redness (a*), and yellowness (b*) was measured by a reflectance colorimeter by using illuminant source C at 2º setting. The colorimeter was calibrated throughout the study using a standard white ceramic tile. Color was evaluated in duplicate at 90º angle to each other from the middle part of the medial (bone side) surface of the left breast, in an area free of obvious color defects, bruises, and blood spots.

3.3.1.3 Ultimate pH measurement

Ultimate pH (pH_u, probe) was measured at approximately 30 h post-mortem. Samples were measured in duplicate by inserting a portable pH meter (Hanna H1 9025 microcomputer pH meter, N. Highland, CA) along with a temperature probe into the cranial part of each right breast sample. After these measurements, all samples were vacuum packed in pre-labelled bags, frozen quickly and held at -30ºC until further testing. The pH_u was also determined using a slurry method in which 5 g of sample was homogenized in 20 mL of deionized water using a Polytron homogenizer (PT 3100,

Kinematica AG, Littau, Switzerland) at 14,600 rpm for 30 sec and the pH of homogenate was measured using a pH meter calibrated at pH 4.0 and 7.0 (Accumet 915 pH meter, Fisher Scientific, Ottawa, ON) equipped with an Accumet pH electrode (Accumet, Fisher Scientific, Nepean, ON).

3.3.1.4 Thaw loss

Thaw loss was measured on the left breast meat samples by thawing the samples overnight at 4ºC. Thaw loss was calculated as the difference between the weight of sample prior to freezing and weight of sample after thawing and blotting and was expressed as percent weight loss.

3.3.1.5 Cook Loss

For the evaluation of cook loss the intact left breast fillets (*Pectoralis* major) were individually vacuum packaged and cooked to an internal temperature of 75 ± 1ºC in an 80 ± 0.5ºC water bath for 25-35 min depending on the sample size. The cooked fillet samples were cooled in cold water for 20 min prior to blotting and weighing. Samples were wrapped at this time and stored at 4ºC overnight for shear force testing the following day. Cook loss was determined as a percentage of weight lost during cooking.

3.3.1.6 Shear Force

Ten rectangular blocks of 1 cm^2 cross section with fibre direction parallel to a long dimension of 2-3 cm were cut from each cooked fillet for Warner Bratzler shear force determination (Fernandez et al., 2001). Shear force was determined using a TMS-Pro Texture analyzer (model 2R1087, Food Technology Corp. Sterling, VA) equipped with a Warner Bratzler shear blade, which cut the sample perpendicular to the fibre direction. Shear force was calculated as the average shear force from the 10 samples.

3.3.1.7 Water binding capacity and pellet cook yield

Water binding capacity (WBC) and pellet cook yield (PCY) was measured on ground breast fillet to assess meat WHC for further processing purposes. WBC and PCY were determined on each ground breast (*Pectoralis* major) fillet using a

modification of the procedure proposed by Mallia et al. (2000) and Van Laack et al. (2000). These modifications allowed a more practical and reproducible method. Right breast fillets were ground for 20 sec using a kitchen blender (12-speed Osterizer Blender, Oster 6650, Mexico City, Mexico). For each breast fillet 5 g was weighed in duplicate into centrifuge tubes, then 8 mL of 0.3 M cold NaCl (4ºC) solution was added and the mixture was vortexed for 15 sec (Fisher Vortex Genie 2^{TM}, Bohemia, NY). Following incubation for 4 h at 4ºC, samples were centrifuged at 7000 × g for 15 min at 4ºC. After centrifugation the supernatant was discarded and tubes were inverted to drip for 15 min. Then tubes were weighed and moisture uptake was calculated as follows:

$$WBC\,(\%) = \frac{\text{pellet weight} - \text{sample weight}}{\text{sample weight}} \times 100\,.$$

Tubes were then heated in an 80ºC water bath for 20 min. Following 20 min inversion while cooling, tubes were reweighed and pellet cook yield was determined as follows:

$$PCY\ (\%) = \frac{\text{weight cooked pellet}}{\text{sample weight}} \times 100$$

3.3.2 Classification of samples into quality groups.

Breast meat samples were categorized into dark, firm, and dry (DFD) (pH> 6.1 and L*< 46.0), normal (5.7≤pH≤6.1 and 46.0≤L*≤53), and pale, soft, and exudative (PSE) (pH<5.7 and L*>53.0) groups. These groupings were adopted from Barbut et al. (2005) and Bianchi et al. (2005) in order to evaluate the effect of environmental temperature during transportation on the incidence of DFD and PSE in broiler breast meat.

3.3.3 Statistical analysis

Temperature and humidity data were imported to an Excel spreadsheet for analysis. Tecplot software (Version 10, Tecplot Inc, Bellevue, WA) was used to estimate the average temperature and humidity of non-sensored grids within the

transport vehicle. Furthermore, data was analyzed using a one-way ANOVA general linear model procedure (GLM) of SAS (SAS Institute, Cary, NC) with the exposure temperature as the variable in the model. Means were separated using the Duncan's multiple range test option of the GLM procedure. Pearson's correlation coefficients (**r**), regression model (R^2), and probabilities were generated using the correlation procedures of SAS. A chi square ($\chi 2$) analysis (Excel version 2003) was used to determine if the number of PSE and DFD samples per temperature group were significantly different at $P < 0.05$.

3.5 Results and Discussion

Birds were grouped into four categories based on the average temperature in their immediate surroundings throughout the transportation as follows: ≤0; 0-10, 10-20 and >20ºC with 86, 159, 143 and 134 birds per group respectively. This grouping method was chosen because the overall temperature differences within each test run were very broad as shown in Table 3.1. Meat quality measures for each temperature classification are shown in Table 3.2. Useable data were obtained on 522 of the potential 540 test birds. None of the test birds died due to transport, but 18 birds were excluded from the study due to condemnation of these birds at the processing plant or loss of bird wing bands during processing and chilling.

Table 3-1 Range of temperature (ºC) within the six drawers for each transportation experiment conducted at a different ambient temperature.

Ambient temperature (ºC)	Min temperature (ºC)	Max temperature (ºC)
-27	-16.3	10.8
-17.4	-13.6	18.3
-22.4	-16.2	14.3
-5	-0.4	22.0
+4	7.8	27.1
+11	13.8	29.6

Table 3-2 Effect of temperature during transportation on breast meat quality properties of broilers.

Property[1]	Temperature Groupings				*P*-value
	≤0ºC	0 <T≤10	10 <T≤ 20	20 <T≤30	
Body temperature (ºC)	39.68±1.3[c]	40.22±1.0[b]	40.46±0.8[a]	40.66±0.6[a]	<0.0001
Grid temperature (ºC)	-7.19±5.0[d]	5.50±2.9[c]	15.23±3.0[b]	24.84±2.6[a]	<0.0001
RH (%)	76.14±6.7[a]	70.36±10.2[b]	53.29±6.7[c]	45.83±4.4[d]	<0.0001
pH_u	5.98±0.2[a]	5.91±0.2[b]	5.91±0.2[b]	5.84±0.2[c]	<0.0001
L*	50.41±3.0[b]	51.57±2.0[a]	51.21±1.8[a]	51.76±2.3[a]	<0.0001
a*	3.79±0.9[a]	3.28±0.8[b]	3.18±0.8[b]	2.75±0.7[c]	<0.0001
b*	1.96±1.7[ab]	2.26±1.3[a]	1.83±1.4[b]	2.03±1.4[ab]	0.0748
Drip loss (%)	0.46±0.1	0.49±0.3	0.48±0.2	0.46±0.2	0.6727
Thaw Loss (%)	1.11±0.5[b]	1.20±0.5[b]	1.18±0.5[b]	1.41±0.5[a]	<0.0001
Cook Loss (%)	14.53±2.2[ab]	14.73±1.9[a]	14.14±2.1[b]	14.98±2.3[a]	0.0085
Shear force (kg)	14.89±2.4	14.63±2.4	14.11±2.6	14.19±2.5	0.0594
WBC (%)	31.45±11.1[a]	28.09±9.9[b]	26.46±10.6[b]	23.06±10.0[c]	<0.0001
PCY (%)	97.14±13.6[a]	91.75±8.6[b]	88.79±10.4[c]	84.99±8.9[d]	<0.0001
n birds/group	86	159	143	134	

[a-d] Means±SD within a row lacking a common superscript differ significantly ($P < 0.05$).

[1]RH= relative humidity, pH_u = ultimate pH (measured by slurry method), L*=lightness, a*= redness, b*= yellowness, WBC= water binding capacity, PCY= pellet cook yield.

Birds used for this study were the first that went through slaughter in the processing plant, however, it was not possible to control the lairage duration since the arrival time to the plant was slightly different based on farm distance and loading time, which lead to a 30 min to 2 h lairage for the test birds. Effect of lairage time was not accounted for in this study, but should have little impact on overall results. It was previously shown that 4 h of lairage following transport had no significant effect on initial pH, color, and shear value of breast meat (Kannan et al. 1997). Warriss et al.

(1999) reported a slight increase in the pH_u of breast meat of transported broiler chickens with one or more h of lairage, but that difference was much smaller than the changes in pH_u reported here. Temperature in the immediate space surrounding each bird showed a significant negative correlation (r = -0.80) with RH. The grids with higher temperatures had significantly ($P < 0.05$) lower RH and vice versa. This is due to the fact that cool air cannot hold as much moisture, therefore RH is higher.

Exposure to harsh environmental temperatures during transportation could affect the body temperature of birds, which is normally between 40.5 and 42.5ºC. In this study the birds transported at temperatures below 0ºC exhibited a significant ($P < 0.0001$) drop of 0.8ºC in their core body temperatures (39.7ºC) compared to the birds exposed to temperatures between 10 and 20ºC and above 20ºC, which had similar core body temperatures of 40.5 and 40.7ºC respectively. However, birds transported at temperatures between 0 and 10ºC, also showed a significant small drop of 0.3ºC in their core body temperature (40.2ºC) compared to warmer temperatures tested (Table 3.2). The relationship between environmental temperature and rectal and deep body temperature has been previously studied in both heat-stressed and cold-stressed birds (Hunter et al., 1999; Sandercock et al., 2001; Berri et al, 2005). Berri et al. (2005) reported that an environmental condition of 35ºC and RH of 60% caused a 1.5ºC increase in rectal temperature, and similarly Sandercock et al. (2001) reported an increase of 1.6 ºC in deep body temperature of birds exposed to temperature of 32.5ºC and RH of 67%. Hunter et al. (1999) showed a 2.1ºC drop in rectal temperature of the birds exposed to 12ºC during transportation (207 ± 48 min), while those exposed to temperatures of 0 and 4ºC showed a further drop of 10ºC in rectal temperature. Furthermore, exposure to -4ºC showed a maximum and potentially lethal decrease of 14.2 ± 5.5ºC in rectal temperature of the cold-stressed birds (Hunter et al., 1999). Our present study showed that cold environment temperatures below 10ºC during transport (3-4 h) would also result in a significant reduction in core body temperature of broiler chickens, yet to a much smaller extent than those reported by Hunter et al. (1999). However, it should be noted that the temperatures reported by Hunter et al. (1999) were average of two temperatures, one was measured prior to transport and another right after

the journey, whereas in this study we reported the average temperature over the entire journey (measured every minute).

Breast meat pH and color values are affected by many pre-slaughter factors, including environmental temperature during transportation. In this study the pH_u for breast meat of birds exposed to temperatures below 0ºC was significantly higher ($P < 0.0001$) (pH = 5.98) compared to pH_u for breast meat of birds exposed to temperatures between 0 and 20ºC (pH_u = 5.91) (Table 2). The pH_u of breast meat was significantly lower ($P < 0.0001$) by ~0.1 unit for birds exposed to temperatures above 20ºC with an average pH_u of 5.84 compared to the cooler temperatures tested. This agrees with reports of several studies (Babji et al., 1982; Holm and Fletcher, 1997; Sandercock et al. 1999; Petracci et al. 2001; and Berri et al., 2005) that higher exposure temperatures were associated with lower pH_u. It is suggested that cold temperature exposure during transport causes glycogen depletion in the muscle of the birds due to increased energy consumption to maintain normal body temperature under these conditions. As a result, these birds have less muscle glycogen stores at the time of slaughter to convert to lactic acid and lower the pH of the meat. However, we did not determine the glycogen content in the present study but more studies are being conducted to further test this hypothesis.

It should be noted that the pH_u was measured using two methods in this study. It was found that the pH_u measured using the slurry method exhibited better correlation (r = 0.78) with other meat quality parameters compared to the pH_u measured by the probe method (r = 0.61). In addition, the correlation between these two methods was only 0.80. Therefore, only pH_u data from the slurry pH_u method were used to compare temperature groups in this paper. It is likely the slurry method gave more accurate results because when measuring pH_u using the probe meter, only one spot is being measured within the breast muscle and the location of this spot might not be exactly the same for all the samples. Furthermore, when pH is high and water is held tightly within the muscle, contact with the pH probe electrode may be different than when a piece of muscle is homogenized and the pH of the homogenate is reported as with the slurry method.

Temperature during transportation had a significant influence on the resulting meat color (Table 3.2), where breast meat of birds exposed to temperatures below 0ºC was significantly ($P < 0.0001$) darker (lower L*) and redder (higher a*) than breast meat of birds exposed to temperatures above 0°C. The meat from birds exposed to temperatures between 0 and 20ºC was redder than meat from birds exposed to higher temperatures (> 20ºC). Breast meat lightness from birds exposed to temperatures above 0ºC was similar. No significant difference or trend was observed for b* value (yellowness) of breast meat from birds exposed to different temperatures during transportation.

The trend observed for pH_u values was in agreement with color redness (a*) values, with both breast meat lightness and pH_u being significantly different for those birds exposed to temperatures below 0ºC. Therefore, the likely cut off temperature for significant changes in pH_u and color, which are the two most important quality attributes of breast meat, in this study could be set at 0ºC. It was previously shown that muscle with higher pH_u is able to hold water more tightly, as a result it looks darker and redder due to less light scattering from the surface (Petracci et al., 2004).

There are contradictory reports concerning the effect of pre-slaughter environmental conditions on broiler breast meat color. Babji et al. (1982) and McKee and Sams (1997) reported a reduction in breast meat color (lighter, less red and less yellow) of turkey toms as a result of an increase in holding temperature (38ºC). Similarly, Bianchi et al. (2006) found that breast meat of broilers exposed to temperatures below 12ºC was significantly darker, redder and more yellow compared to those exposed to temperatures between 12 and 18, and above 18ºC. However, Holm and Fletcher (1997) and Sandercock et al. (2001) found no significant effect of the holding temperature on either a* or L* color values of broiler breast meat except for yellowness (b*) that increased as temperature increased. It should be noted that the temperatures studied by Petracci et al. (2001) ranged from 25 to 34ºC and that of Holm and Fletcher (1997) ranged from 7 to 29ºC. Holm and Fletcher (1997) reported that breast meat of birds exposed to 29ºC showed lower pH_u compared to the control (18ºC) and cold (7ºC) temperatures. Although the range of temperatures in the present study is completely

different from the earlier studies a similar trend was observed as the temperatures rise above or fall below the birds' thermoneutral temperatures.

Babji et al. (1982) showed that breast meat of cold-stressed (4ºC for 4 h) and control (21.1ºC for 4 h) tom turkeys had significantly higher myoglobin content compared to heat-stressed turkeys (38ºC for 4 h). However, it is difficult to accept that heat stress prior to slaughter could cause a reduction in total pigment concentrations, but a possible explanation given by these authors was that the lower pigment concentration could be attributed to the rapid rate of post-mortem glycolysis at high temperatures that result in rapid pH decline and decrease in color intensity, and potential denaturation of myoglobin. Color variation plays an important role in consumer acceptance, especially if multiple color fillets are packed with noticeable color differences (Fletcher, 1999a). According to Petracci et al. (2004) breast meat of birds collected in the summer showed significant ($P < 0.05$) decrease in lightness (53.05 vs. 52.79 and 51.31) and increase in redness (2.82 vs. 3.20 and 3.80) and yellowness (3.25 vs. 3.34 and 3.93) values compared to the breast meat of birds collected during autumn and winter. The tendency of the breast meat to show a higher a* value (redder) when the L* value decreases (becomes darker) observed in this study is consistent with previous studies (Van Laack et al., 2000; Petracci et al., 2001; Bianchi et al., 2006).

Drip loss did not show any significant difference based on temperature groupings. Furthermore, cook loss values were similar and no specific trend was observed based on exposed temperature during transportation. However, thaw loss was significantly higher for breast meat of the birds exposed to temperatures above 20ºC in this study. Sandercock et al. (2001) showed a higher drip loss for breast meat of heat-stressed birds (32.5ºC and RH of 67.1 %). Holm and Fletcher (1997) showed a significantly lower cook loss for birds exposed to 29ºC compared to the control (18ºC) and cool temperature (7ºC). Babji et al. (1982) did not find any significant effect of heat-stress (38ºC for 4 h) or cold-stress (5ºC for 4 h) on the subsequent breast meat cook loss of 26 week-old tom turkeys. The contrast in the findings between the present study and previous studies could be explained by the different range of temperatures tested. However, we expected to find lower drip and cook loss values for the birds exposed to

temperatures below 0ºC, since these samples showed higher ultimate pH. Meat with high ultimate pH is able to hold more water since the contractile proteins are further from their isoelectric points, there is higher net charge on the proteins and greater intracellular spaces available for water and also there are more binding sites to attract water (Barbut, 1993; Van Laack et al., 2000). Based on results of our present study, transportation of birds, generally, did not show much effect on WHC of the intact breast meat when the temperature falls below 0ºC.

While WHC is an important quality attribute of the intact meat, WBC plays an essential role in further processed products. WBC is closely related to the pH_u of the meat (Van Laack et al., 2000) and therefore was affected by environmental temperature prior to slaughter in a similar way as pH_u. Temperatures below 0ºC resulted in a significantly higher ($P<0.0001$) breast meat WBC (31%) compared to the breast meat of birds exposed to temperatures between 0 and 20ºC (26-28%), which in turn had significantly higher WBC than meat from those exposed to temperatures above 20ºC (23%). Babji et al. (1982) showed reduced WBC from breast meat of tom turkeys exposed to heat-stress (37.8ºC) compared to those exposed to the cold (4.4ºC) and control (21.1ºC) temperatures that were not different from each other. Sandercock et al. (1999) and Petracci et al. (2001) also showed reduced WBC for birds exposed to heat-stress prior to slaughter. However, the range of temperature tested is different between these studies. The highest temperature (24.5ºC) reported in our present study was considered as the cool holding temperature in the study by Petracci et al. (2001). Pellet cook yield (PCY) was significantly ($P < 0.0001$) different between all temperature groupings. The highest PCY was found in breast meat of broiler chickens exposed to temperatures below 0ºC. Therefore, sub-zero temperatures resulted in higher WBC, PCY and darker breast meat color, which is strongly related to the higher ultimate pH observed for the breast meat of these birds. There was a greater effect of pH_u on water binding properties compared to WHC as the charged groups on the ground muscle were able to interact more fully with the excess water provided, whereas there would be steric hinderances in intact muscle so that the WHC of intact muscle appeared less sensitive to the ranges of pH found. In the present study, breast meat of birds exposed to temperatures below 0ºC tended ($P = 0.06$) to have lower shear values compared to the

breast meat of other temperature groupings. Several studies (Froning et al., 1978; Babji et al., 1982; Petracci et al., 2001; Sandercock et al., 2001) have reported a reduction in tenderness with increase in exposure temperature.

Using the Pearson correlation option of SAS, a significant ($P < 0.0001$) negative correlation was found between pH_u and L* (r = -0.62), b*(r = -0.60), drip loss (r = -0.26), thaw loss (r = -0.56), and cook loss (r = -0.54) values, in addition a significant ($P < 0.0001$) positive correlation was found between pH_u and a* (r = 0.20), WBC (r = 0.73), and PCY (r = 0.69) of the broiler chickens breast meat (Table 3.3). This indicated that breast meat with higher pH_u was darker (lower L*), redder (higher a*), and holds more water compared to the lower pH_u breast meat. Furthermore, pH_u, L*, and WBC values showed a significant ($P < 0.0001$) correlation with all other meat quality parameters measured, suggesting that these parameters are reliable measure of broiler breast meat quality characteristics, which is in agreement with several previous studies (Owens and Sams, 2000; Van Laack et al., 2000; Bianchi et al., 2005).

The temperatures of the immediate surroundings of the broiler chickens during transportation were shown to affect meat quality parameters. Significant ($P <0.0001$), but low correlations were found between temperature during transportation and breast meat pH, L*, a*, thaw loss, WBC and PCY parameters (Table 3.3). Exposure of birds to colder temperatures resulted in breast meat with higher pH_u (r = -0.24), lower L* (r = 0.17), higher a* (r = -0.39), lower thaw loss (r = 0.17), and higher WBC (r = -0.27) and PCY (r = -0.39). These results are consistent with those presented previously by Froning et al. (1978), Babji et al. (1982), Holm and Fletcher (1997).

Quality parameters of samples classified as DFD, normal and PSE breast meat are shown in Table 3.4. The incidence of DFD in breast meat was observed only with exposure temperatures below 0ºC. However, the frequency of DFD incidence was about 1.5% overall and less than 8% even when the surrounding temperature during transportation was less than 0ºC (Figure 3.2). The incidence of PSE was significantly ($P < 0.05$) higher at temperatures above 20ºC (13%) compared with temperatures below 0ºC (4%) (Figure 3.2). This finding agrees with several other studies that reported higher incidence of PSE breast meat in turkey and chicken as a result of seasonal heat-

stress (McKee and Sams, 1997; Owens and Sams, 2000; Petracci et al., 2004) and reduced occurrence (5.9%) of PSE breast meat for birds processed during winter (Bianchi et al., 2006).

A high incidence of DFD breast meat was reported by Lesiów et al. (2007) in broilers during the winter season. However, their DFD incidence was reported as 18-34%, when L* was less than 48 (using Minolta spectrophotometer with the same setting as the present study) and 0-10% when L* was less than 46 as the criteria for sorting DFD meat. Therefore, this report agrees with the present study since the boundary chosen in our present study was 46 for L* value, although there is no indication of the temperature during winter transportation in the Lesiów et al. (2007) study.

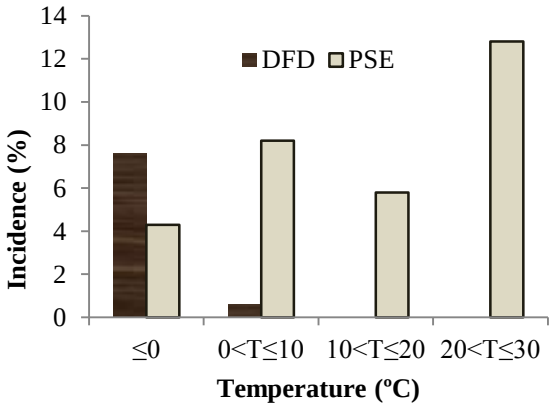

Figure 3-2 Incidence (%) of dark, firm and dry (DFD) and pale, soft, and exudative (PSE) broilers chicken breast meat under different environmental temperatures during transportation. T= temperature.

Table 3-3 Pearson correlation coefficients between breast meat quality parameters (n=522).

Property[1]	GT	RH	pH_u	L*	a*	b*	TL	CL	WBC	PCY
GT	1.00									
RH	-0.83**	1.00								
pH_u	-0.24**	0.13**	1.00							
L*	0.17	0.08	-0.62**	1.00						
a*	-0.39**	0.26**	0.20**	-0.47**	1.00					
b*	-0.02	0.08	-0.60**	0.64**	-0.16**	1.00				
TL	0.17	-0.10	-0.56**	0.53**	-0.19**	0.39**	1.00			
CL	-0.03	0.07	-0.54**	0.56**	-0.26**	0.44**	0.50**	1.00		
WBC	-0.27	0.16**	0.73**	-0.68**	0.28**	-0.53**	-0.65**	-0.57**	1.00	
PCY	-0.39**	0.30**	0.69**	-0.65**	0.32**	-0.47**	-0.57**	-0.56**	0.90**	1.00
DL	-0.04	0.06	-0.26**	0.33**	-0.06	0.23**	0.43**	0.30**	-0.38**	-0.30**

*$P \leq 0.05$, **$P \leq 0.001$.

[1]GT= grid temperature (temperature surrounding individual birds); RH= relative humidity; pH_u= ultimate pH (measured by slurry method); L*=lightness; a*= redness; b*= yellowness; TL= thaw loss; CL= cook loss; WBC= water binding capacity; PCY= pellet cook yield; DL= drip loss.

Table 3-4 Meat quality parameters and incidence of dark, firm, and dry (DFD), normal and pale, soft, and exudative (PSE) meat within the population (n=522)

Property[1]	Meat quality group[2]			*P*-value
	DFD	Normal	PSE	
Number of birds/group	8	472	42	
Body temperature (ºC)	37.87±1.8[b]	40.35±0.9[a]	40.37±1.2[a]	<0.0001
Grid temperature (ºC)	-11.32±6.6[b]	11.10±11.2[a]	14.62±9.6[a]	<0.0001
RH (%)	78.43±7.1[a]	60.22±13.8[b]	58.22±15.3[b]	0.0007
pH_u	6.43±0.2[a]	5.92±0.2[b]	5.62±0.1[c]	<0.0001
L*	43.34±2.0[c]	51.18±1.9[b]	54.58±1.4[a]	<0.0001
a*	4.91±0.9[a]	3.23±0.8[b]	2.56±0.7[c]	<0.0001
b*	-1.33±0.9[c]	1.94±1.3[b]	3.77±0.9[a]	<0.0001
Drip loss (%)	0.35±0.1[b]	0.46±0.2[b]	0.64±0.3[a]	<0.0001
Thaw loss (%)	0.46±0.2[c]	1.19±0.5[b]	1.86±0.6[a]	<0.0001
Cook loss (%)	10.41±2.2[c]	14.51±2.1[b]	16.35±1.6[a]	<0.0001
Shear (kg)	13.93±2.3[b]	14.24±2.4[b]	16.51±2.9[a]	<0.0001
WBC (%)	50.78±14.6[a]	27.93±9.2[b]	10.90±6.9[c]	<0.0001
PCY (%)	125.44±24.4[a]	90.55±9.1[b]	78.21±7.3[c]	<0.0001
Incidence of total (%)	1.5	90.4	8.1	

[a-c] Means±SD within a row lacking a common superscript differ significantly ($P < 0.05$).

[1]RH= relative humidity, pH_u = ultimate pH, L*=lightness, a*= redness, b*= yellowness, WBC= water binding capacity, and PCY= pellet cook yield.

[2]DFD (pH> 6.1 and L*< 46.0), Normal (5.7≤pH≤6.1 and 46.0≤L*≤53), and PSE (pH<5.7 and L*>53.0).

The DFD breast meat samples exhibited significantly higher ($P < 0.0001$) pH_u, a* (redder), WBC and PCY; but lower L* (darker), b* (less yellow), thaw loss, and cook loss compared to normal and PSE breast meat samples (Table 4). The dark fillets

exhibited significantly higher ($P < 0.0001$) moisture uptake (50.8% ± 14.6) compared to the normal (27.9% ± 9.2) and the pale (10.9% ± 6.9) samples. These characteristics of DFD meat was previously reported by several studies (Mallia et al., 2000; Barbut et al., 2005). However, Barbut et al. (2005) did not find significant difference in cook loss and brine uptake between DFD and normal samples. Furthermore, in our present study DFD samples exhibited lower drip loss and lower shear values (more tender) compared to the PSE samples, but were not significantly different from the normal samples. The pale breast meat fillets had significantly lower ($P < 0.0001$) pH and WBC and higher ($P < 0.0001$) shear values compared to normal and DFD breast meat. These characteristics of PSE breast meat are previously reported by several studies (Fletcher, 1999b; Van Laack et al., 2000; Petracci et al., 2004). Therefore, the present study shows a dominant effect of pH and L* on all meat quality characteristics, which were influenced by transport temperature of the broiler chickens.

3.6 Conclusion

In conclusion, the results of this study demonstrate that the immediate temperature surrounding broiler chickens during pre-slaughter transportation may affect subsequent breast meat quality by causing variations in breast meat color, ultimate pH and water binding capacity. The water holding capacity of ground meat with added brine (0.3 M NaCl) was shown to be very responsive to pH_u and increased as pre-slaughter temperature decreased, while the effect of transportation temperature on the water holding capacity of intact breast meat was not significant. A higher occurrence of PSE meat was observed as temperature surrounding the broiler chickens during transportation increased from sub-zero temperatures to above 20ºC. Furthermore, an 8% incidence of DFD breast meat was observed for birds exposed to temperatures below 0ºC during transportation. DFD breast meat was associated with a significantly darker color, higher pH_u and higher water holding capacity compared with normal breast meat. In addition, birds exposed to temperatures below 0ºC had significantly lower core body temperatures compared to transport temperatures above 10ºC.

3.7 Connection to the Next Study

A second study was carried out to further investigate effect of transportation on breast meat quality during cold conditions, specifically temperatures below -10ºC, which are very common in Saskatchewan winters. Many other factors, that were not accounted for in the first study might have affected breast meat quality, such as bird age or size and gender, lairage duration and transport duration. Due to variation in temperature during Saskatchewan winters, and also variation in temperature within a transport truck, it was very difficult to attain certain temperatures during actual transportation under commercial conditions. In addition, controlling lairage duration was very difficult in the previous study as the trailers arrived at different times to the slaughter plant due to different distances from farm to the slaughter plant and time of loading. In addition welfare assessment could not be performed properly at the farm or during lairage at the slaughter plant. Therefore, based on all the difficulties dealing with actual transportation trials and in order to minimize some of the uncontrollable variables during actual transportation, such as air flow and stopping of the trailer on the way to the slaughtering plant, an environmental chamber was created by Dr. Crowe's group in the Department of Agricultural and Bioresource Engineering to simulate transport conditions under desired temperatures, which further enabled us to control parameters such as age and gender of birds and duration of lairage prior to slaughter.

4. EFFECT OF ACUTE COLD EXPOSURE, AGE, GENDER AND LAIRAGE ON BROILER BREAST MEAT QUALITY

4.1 Abstract

The effect of acute cold exposure was assessed on bird physiology, muscle metabolites and meat quality from 360 male and female broilers of 5 and 6 wk of age, exposed for 3 h to temperature ranges of -18 to -4ºC and control of +20ºC using a simulated transport system followed by 0 or 2 h of lairage. Core body temperature (CBT) was recorded, and the microclimate temperature and relative humidity surrounding individual birds were monitored. Birds were classified based on the temperature in their immediate surroundings. Exposure to temperatures below 0ºC resulted in a decrease ($P < 0.05$) in blood glucose and an increase in live shrink. During the 3 h of exposure to -8ºC or lower, CBT dropped substantially. Temperatures below -14ºC caused lower glycolytic potential and lactate concentrations. Five wk birds showed darker and redder breast meat with higher ultimate pH (pH_u), lower cook loss and higher processing cook yield (PCY) at temperatures below -8ºC compared to warmer temperatures and compared to 6 wk birds exposed to similar temperatures. No difference in meat quality was observed between the two ages at temperatures below -14ºC. Males had a greater drop in CBT and breast meat with higher pH_u compared to females. The 2 h lairage resulted in darker breast meat with higher pH_u at exposure temperatures below -14ºC and higher water binding capacity and PCY at temperatures below -11ºC. A high (> 57%) incidence of dark, firm and dry (DFD**;** pH > 6.1 and L* < 46) breast meat was observed at temperatures below -14ºC. Two h lairage resulted in an

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additional 20% increase in DFD incidence at temperatures below -8ºC. Results of this study showed that older birds coped better with extremely cold conditions compared to younger birds. Furthermore, it would be beneficial to limit the length of lairage prior to processing following acute cold exposure, to improve welfare and reduce meat quality defects.

4.2 Introduction

Several studies have described the effects of pre-slaughter treatments including feed withdrawal, handling, transport and environmental temperature on metabolic and stress related parameters, as well as subsequent meat quality measures of poultry breast meat (Lee et al., 1976; Froning et al., 1978; Babji et al., 1982; Holm and Fletcher, 1997; Debut et al., 2003). Stress prior to slaughter could alter muscle glycogen content, which is the main energy reserve affecting the rate and extent of pH drop in muscle (Rammouz et al., 2004 a, b; Berri et al., 2007), and ultimate pH (pH_u) is the main factor affecting all quality attributes including color and water holding capacity (WHC).

Cool temperatures (4, 5 and 7ºC) prior to slaughter are reported to cause a moderate increase in pH_u and result in darker color breast meat with better functional properties (Froning et al., 1978; Babji et al., 1982; Holm and Fletcher, 1997). It was shown in Chapter III that transportation of birds at exposure temperatures below 0ºC for 3-4 h prior to slaughter resulted in breast meat with darker color, higher pH_u and higher water binding capacity causing an increase in the incidence of dark, firm, dry (DFD) breast meat, but had no effect on breast meat tenderness. On the other hand, no significant effect of cold exposure (4ºC) or extreme cold exposure (-20ºC) for a duration of 6 h was reported on breast meat tenderness, muscle glycogen level or rate of glycolysis from 8 wk old female broiler chickens by Lee et al. (1976).

The objective of the present study was to investigate the effect of exposure of broiler chickens to extreme cold microclimate temperatures, similar to environmental conditions during a Canadian winter, on physiological parameters (core body temperature (CBT), live shrink and blood glucose), breast muscle metabolites (glycolytic potential) and breast meat quality (pH, color and WHC) considering

variables such as age and gender of the birds as well as rest prior to slaughter (lairage). In addition, incidence of DFD breast meat in relation to these parameters was investigated.

4.3 Materials and Methods

This project was approved by the University Committee on Animal Care and Supply of the University of Saskatchewan, which is subject to the Canadian Council on Animal Care. An experimental test chamber, capable of simulating conditions within a transport vehicle was used in this study. The chamber used a fan to pull air from outside the building through a digitally controlled heater and two partitioned drawers placed on top of each other in the chamber, similar to their orientation in a transport vehicle. A total of 360 broilers with 178 females and 182 males, were tested: 180 at 35-37 days (5 wk) and 180 at 40-42 days (6 wk). Feed was removed 7 h prior to the start of each trial, which led to 10 and 12 h of feed deprivation prior to slaughter for birds in the 0 and 2 h lairage treatments, respectively. Birds were wing banded, weighed, and orally dosed with mini temperature loggers (Thermocron iButtons® DS1922L iButtonÂ®, Maxim Integrated Products, CA) into the proventriculus to measure core body temperature (CBT) during the trial. The loggers were set to record temperature once each minute and were retrieved after birds were euthanized at the end of the trial. For each treatment, thirty birds (balanced between genders) were exposed to one of the assigned temperatures (-18, -15, -12, -8 or -4, 20ºC) within 2 drawers. Due to the weather changes temperature of -4 could not be attained for the 6 wk birds, therefore these birds were exposed to -8 ºC. The drawers were partitioned into 15 individual 112 x 71cm compartments each containing one bird, and placed on top of each other in the chamber. The temperature and humidity of each bird location was monitored once each minute with mini loggers (Hygrocon iButton® DS19223 iButtonÂ®, Maxim Integrated Products, CA). Temperature and RH were interpolated for the center position of each grid space using mapping software (Version 10, Tecplot Inc, Bellevue, WA).

At the end of the 3 h exposure the drawers were removed from the chamber. Birds from one of the two drawers, randomly chosen, were weighed and blood samples were collected within 10 min (0 h lairage); birds from the other drawer were allowed to

remain in the mesh covered drawer at room temperature for 2 h prior to weighing and blood sampling. Live shrink was calculated as the difference between the live weights measured before and after the 3-h treatment. Blood glucose levels were measured using a glucose kit (i-STAT^® 1 Handheld Clinical Analyzer", Heska Inc., Loveland, CO). Birds were sacrificed by hand-cutting the carotid artery and jugular veins on both sides of the neck after electrical stunning (VS 200 stunner knife, Midwest Processing System, Edina, MN) for 5 sec adjusted to 40-50 V and 30-50 mA settings. Following a 2 min exanguination period, birds were scalded in a 66-68ºC water tank for 30s and defeathered in an automatic feather picker (Pro model K7080, Featherman Equipment, MO) for another 30 s. Liver samples were collected immediately following evisceration (5-8 min post-mortem) by removing the smallest lobe of the liver, which was then frozen in liquid nitrogen and stored at -80ºC. Carcasses were placed into a 0°C chill tank 20 min post-mortem for 40 min, with frequent agitation and then packed on ice and stored at 4°C until deboning.

4.3.1 Meat quality measurements

The carcasses were weighed, and manually deboned at 6-8 h post-mortem. The left and right breast meat pieces (*Pectoralis* major) were removed for evaluation of quality parameters, and core samples frozen at 30 h post-mortem were used to measure lactate concentration and glycolytic potential of the breast meat as described below.

4.3.1.1 Color

Meat color was evaluated at 30 h post-mortem using a Minolta Chroma meter (RC-400). The CIE system color profile of lightness (L*), redness (a*), and yellowness (b*) was measured by a reflectance colorimeter using illuminant source C at the 2º setting. The colorimeter was calibrated throughout the study using a standard white ceramic tile. Color was evaluated in duplicate at 90º angle to each other from the middle part of the medial (bone side) surface of the left breast, in an area free of obvious color defects, bruises, and blood spots.

4.3.1.2 Ultimate pH

The pH_u was determined using a slurry method in which 5 g of each sample collected at 30 h post-mortem was homogenized in 20 mL of deionized water using a Polytron homogenizer (PT 3100, Kinematica AG, Littau, Switzerland) at 14,600 rpm for 30 s. The pH of the homogenate was measured using a pH meter calibrated at pH 4.0 and 7.0 (Accumet 915 pH meter, Fisher Scientific, Ottawa, ON) equipped with an Accumet pH electrode.

4.3.1.3 Water holding capacity (WHC)

WHC of breast meat was assessed by measuring its drip, thaw and cook losses as per Dadgar et al. (2010) (Chapter III). Drip loss was expressed as the percent of weight lost during 24 h of simulated retail display (stored at 4ºC). Thaw loss was expressed as the percentage of weight lost during thawing overnight at 4ºC. Cook loss was expressed as the percentage of weight lost during cooking of the intact breast fillets to an internal temperature of 75 ± 1ºC in an 80 ± 0.5ºC water bath for 25-35 min (depending on the sample size).

4.3.1.4 Shear force

Ten rectangular blocks of 1 cm^2 cross section, with fibre direction parallel to a long dimension of 2-3 cm, were cut from each cooked fillet for Warner Bratzler shear force determination (Fernandez et al., 2001). Shear force was determined using a TMS-Pro Texture analyzer (Model 2R1087, Food Technology Corp., Sterling, VA) equipped with a Warner Bratzler shear blade, which cut the sample perpendicular to the fibre direction. Shear force was calculated as the average shear force from the 10 samples.

4.3.1.5 Water binding capacity (WBC) and processing cook yield (PCY)

WBC and PCY were determined according to Dadgar et al. (2010) (Chapter III) on ground breast fillet. For each breast fillet, 5 g was weighed in duplicate into centrifuge tubes, then 8 mL of 0.3 M cold NaCl (4ºC) solution was added and the

mixture was vortexed for 15 sec (Fisher Vortex Genie 2^{TM}, Bohemia, NY). Following incubation for 4 h at 4ºC, samples were centrifuged at 7000 × g for 15 min at 4ºC. After centrifugation the supernatant was discarded and tubes were inverted to drip for 15 min. Then tubes were weighed and moisture uptake was calculated as follows:

$$WBC\ (\%) = \frac{\text{pellet weight} - \text{sample weight}}{\text{sample weight}} \times 100.$$

Tubes were then heated in an 80ºC water bath for 20 min. Following 20 min inversion while cooling, tubes were reweighed and processing cook yield was determined as follows:

$$PCY\ (\%) = \frac{\text{weight cooked pellet}}{\text{sample weight}} \times 100$$

4.3.1.6 Muscle metabolites

Total carbohydrate (glucose, glucose 6-phosphate (G6P) and glycogen) was measured using the hydrochloric acid method modified from Passonneau and Lauderdale (1974). Immediately after removal from the freezer, one gram of minced, frozen breast or liver samples was homogenized with 10 mL of 0.03 N HCl for 30 s at full speed using a Polytron homogenizer (PT 3100, Kinematica AG, Littau, Switzerland). The homogenate was placed in a boiling water bath for 5 min to stop any enzymatic reactions. One mL of homogenate was boiled with 2 mL of 1 N HCl for 3 h, and the mixture neutralized with 2 mL of 1 N NaOH following hydrolyzation (Bee, 2002). An aliquot (1 mL) of this sample was centrifuged for 10 min at 12,000 rpm (11,758 x g) (Eppendorf Centrifuge 5415C), and used for total carbohydrate and lactate measurements. Concentration of NADPH produced was measured spectrophotometrically at 340 nm using a Glucose Hexokinase (HK) assay kit (Sigma, GAHK-20).The concentration of NADPH was equivalent to the total carbohydrate in the sample.

$$\text{Glycogen}_{n} + n\text{-}1\,H_2O \xrightarrow{HCl, pH=2} (\text{Glucose})_n$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase, } Mg^{2+},\ pH7.5} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP}^{+} \xrightarrow{\text{G6P dehydrogenase}} 6\text{-phosphogluconolactone} + \text{NADPH} + H^{+}$$

Lactate concentration was measured according to Hartschuh et al. (2002). First, 1.45 mL lactate assay buffer [0.01 g NAD^+, 2 mL glycine buffer, 4 mL deionized water, 0.1 mL lactate dehydrogenase] was added to 50 µL of the sample and then the concentration of the NADH produced was measured spectrophotometrically at 340 nm after 60 min incubation at room temperature.

$$\text{L-(+)-Lactate} + \text{NAD}^{+} \xrightarrow{\text{lactate dehydrogenase}} \text{Pyruvate} + \text{NADH} + H^{+}$$

Glycolytic potential (**GP)** was expressed as moles of lactate (equivalent to actual lactate plus lactate that could be generated from total carbohydrate) per gram of meat (Hartschuh et al., 2002). GP was calculated according to Monin and Sellier (1985) as follows:

GP= 2 (glucose+G6P+glycogen) + lactate

4.3.2 Classification of samples into quality groups

Breast meat samples were categorized into dark, firm, dry (DFD) (pH> 6.1 and L*< 46.0), and normal (5.7≤pH≤6.1 and 46.0≤L*≤53) groups. These groupings were adopted from Barbut et al. (2005) in order to evaluate the effect of environmental temperature during transportation on the incidence of DFD breast meat. There was no sample with pale, soft, and exudative (PSE) (pH<5.7 and L*>53.0) traits among the breast meat samples evaluated.

4.3.3 Statistical analysis

A completely randomized design with a 5 x 2 x 2 x 2 factorial arrangement was employed in this experiment, with 9 birds per treatment combination (n = 360). The model included the main effects of immediate temperature surrounding birds (-17<T≤

-14, -14<T≤-11, -11<T≤-8, -8<T≤0 and 20<T≤22), age (5 or 6 wk), gender (female or male), and lairage (0 or 2 h) at slaughter and the interactions between these factors as the main sources of variation. Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (SAS Institute, Cary, NC) and results were reported as least square means with their standard deviations. Differences among means were evaluated using the Duncan's multiple comparison test option of SAS. Unless otherwise stated, the means were considered different at $P < 0.05$.

4.5 Results and Discussion

Birds were grouped based on the temperature in their immediate surroundings throughout the 3 h exposure as follows: -17<T≤-14, -14<T≤-11, -11<T≤-8, -8<T≤0 and 20<T≤22 with 71, 83, 70, 76, and 60 birds per group, respectively. Bird physiology, muscle metabolites and meat quality parameters were compared based on different temperature groupings. In interpreting these results it should be remembered that the chickens used in this study were individually partitioned within the drawers. Commercial transport systems currently in use allow birds to huddle, which is a significant benefit at low temperatures. The system employed here was designed to accurately control each bird's exposure and measure its response, which would not be possible if the birds were allowed to co-mingle with other birds in the drawer.

4.5.1 Effect of cold exposure on bird physiology

The core body temperature (CBT) of birds decreased during the 3 h exposure at simulated transport temperatures below -8°C for the 5 wk, and below -14°C for the 6 wk birds (Figure 4.1). In a tolerance test to severe hypothermia, the lower lethal body temperature was reported to be ~24°C for hens and ~21°C for cocks of approximately 1 year of age (Sturkie, 1946). A total of 9%, and 23% of the 5 wk birds reached CBT below 24°C when exposed to temperatures below -11 and -14°C respectively. Among the 5 wk birds, one did not survive the extreme cold exposure and several birds

experienced CBT below 20ºC at slaughter. Since the dead bird (male with 0 h lairage) was equipped with an I-button it was included in statistical analysis for CBT and it was considered as missing data for analysis of muscle properties. However, within the 6 wk birds only one bird showed CBT of 21°C at time of slaughter, and CBT of the rest of the birds remained above 30°C during 3 h exposure to temperatures below -14°C (data are not shown). Therefore, 6 wk birds (2.6 kg) could better manage their CBT during the 3 h of simulated transport under cold ambient temperatures compared to 5 wk birds (1.9 kg) (Figure 4.1), which could be related to the larger body size and more feather coverage of 6 wk birds, since body mass play an important role in energy balance (Blem, 2000)

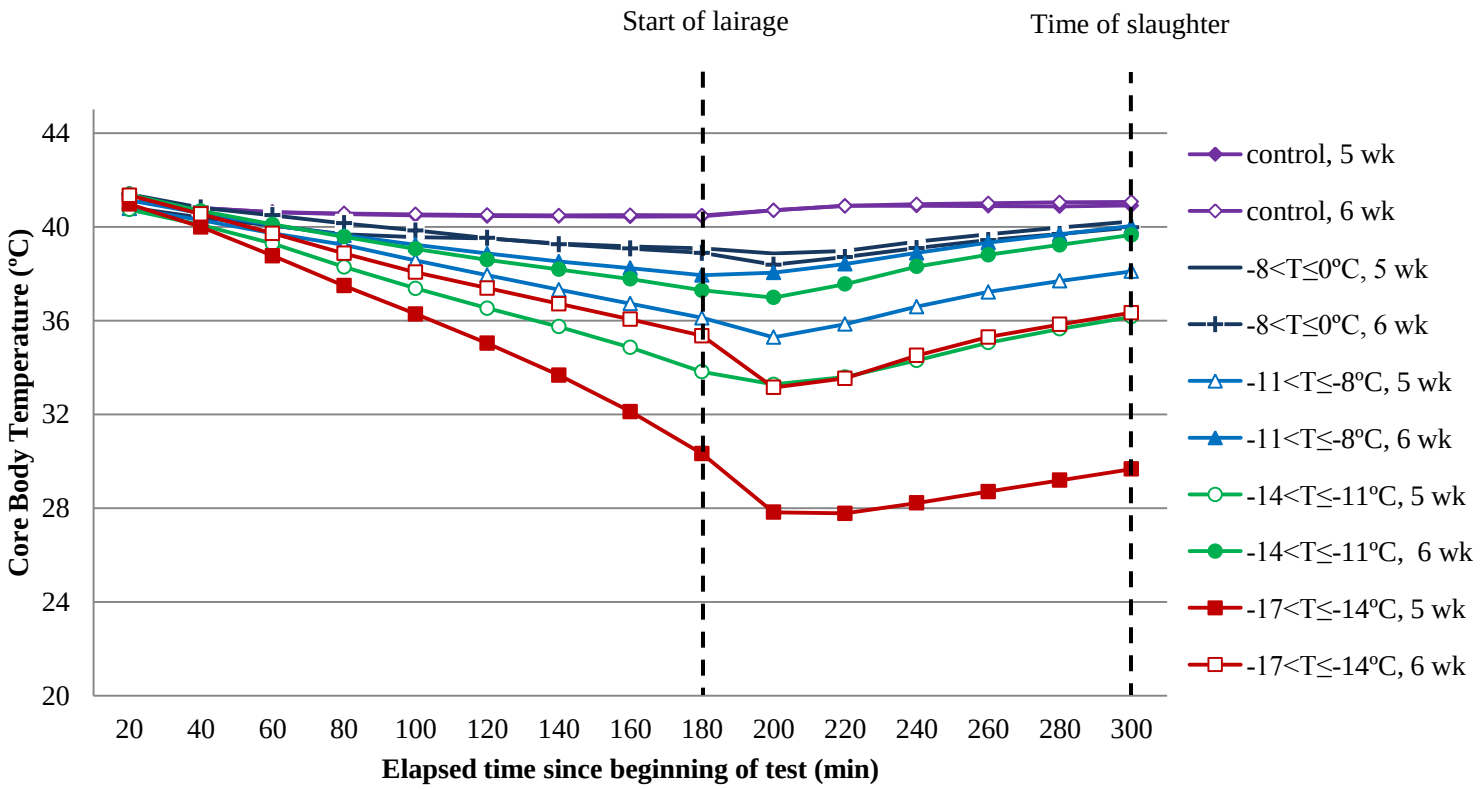


Figure 4-1 Change in core body temperature of birds with time during exposure to various temperatures during simulated transport (0 to 180 min) followed by lairage (120 min).

Hunter et al. (1999) observed a dramatic decrease in rectal temperature (14.2 ± 5.5ºC) of broiler chickens as a result of cold (-4ºC) transport for a duration of 207 ± 48 min. Furthermore, these authors reported that transport of birds at temperatures of 12, 0 and 4ºC also resulted in significant drops in rectal temperature (2.1, 10 and 10ºC respectively) (Hunter et al., 1999). However, in the current study, the CBT of birds during simulated transport of birds at average temperatures of -4ºC showed only a small decrease (Figure 4.1). Difference in results can be explained by the wet transport conditions conducted in the study of Hunter et al. (1999) compared to the dry conditions in this study.

Two h of lairage following acute cold exposure exacerbated the situation for some birds, but the majority of birds were able to recover to their initial CBT after 30 min (Figure 4.1). The ability of birds to return to normal body temperature during lairage following cold exposure is dependent on the physiological status of birds at initiation of lairage.

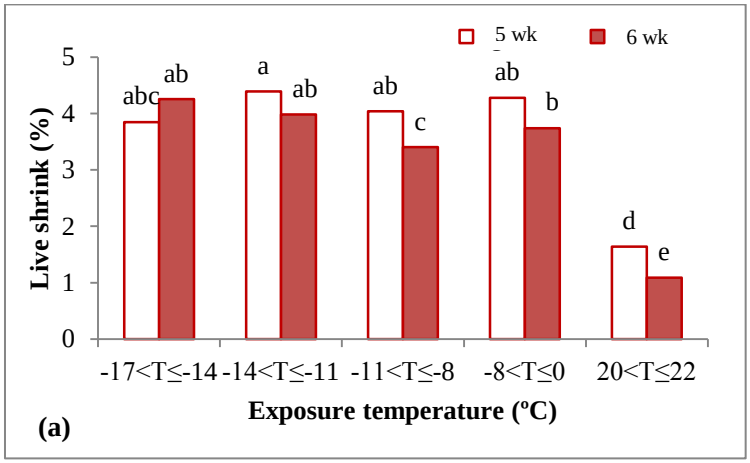
Male broilers (2.5 kg) showed an average CBT of 38.7°C compared to 39°C for females (2.1 kg) (Table 4.1). The higher CBT for females was likely associated with more feather coverage providing a better barrier to cold exposure and higher abdominal fat providing an extra source of fuel compared to males. Greater breast and abdominal fat yield were reported for females despite a similar body weight compared to males (Berri et al., 2007).

Live shrink was found to be higher for birds exposed to any of the cold temperatures tested compared to that of control birds (Table 4.1), which may be related to shivering thermogenesis and excess energy usage during cold exposure in order to maintain homeostasis. Five wk old birds showed higher live shrink compared to 6 wk old birds when exposed to -8 to -11ºC and control temperatures (Figure 4.2), also tended to have higher live shrink at other temperatures tested when it was above -14ºC. The higher live shrink for younger birds could be related to higher proportion of gastrointestinal tract to body mass of the smaller birds compared to the older and bigger birds, but more likely, it was due to the greater difficulties that young birds experienced in coping with the cold conditions due to their greater surface area and less feather

cover than older birds at temperatures above -11ºC, below which no difference in live shrink was observed between the two ages. Birds slaughtered immediately after treatment showed 3.4% live shrink compared to 3.7% for birds given 2 h of lairage. The increase in live shrink with lairage could be related to extended feed withdrawal and usage of energy through catabolizing body tissue in order to recover CBT (Table 4.1). No effect of gender was observed on live shrink of broiler chickens.

Blood glucose concentration was higher for control birds compared to birds exposed to all of the cold temperatures tested (Figure 4.2). Blood glucose concentration showed a gradual decrease with decrease in exposure temperature for both ages (Table 4.1). This probably reflects an increase in energy consumption with decrease in exposure temperature to maintain CBT. The 5 wk birds showed lower blood glucose compared to the 6 wk birds when exposed to temperatures colder than -8ºC (Figure 4.2), whereas blood glucose concentration was higher for the 5 wk control birds compared to the 6 wk control birds. A significant interaction of gender by lairage was observed for blood glucose, where females given 2 h of lairage showed higher (9.2 mmol/L) blood glucose compared to males (8.7 mmol/L), whereas females had lower blood glucose (8.5 mmol/L) compared to males (9.1 mmol/L) when immediately slaughtered after exposure.

Liver glycogen has been reported to reach negligible amounts after several h of feed withdrawals (Nijdam et al., 2005a). In the current study, the overall liver glycogen content measured after 10 and 12 h of feed deprivation for the 0 and 2 h lairage birds, respectively, was very small (15-23 µmol/g). Three way interactions of temperature, age by lairage, and temperature, age by gender were observed for liver glycogen, however these interactions were difficult to interpret, and were mainly related to few outliers (Data not shown). Furthermore, a significant interaction of lairage by gender was observed (Table 4.1) on liver glycogen content at slaughter, where males tended to have higher liver glycogen when given 2 h of lairage compared to females, which might be due to more muscle mass for males (2.5 kg) compared to females (2.1 kg) enabling them to rebuild their liver glycogen reserve (data not shown), or simply because they are able to recover faster than females.



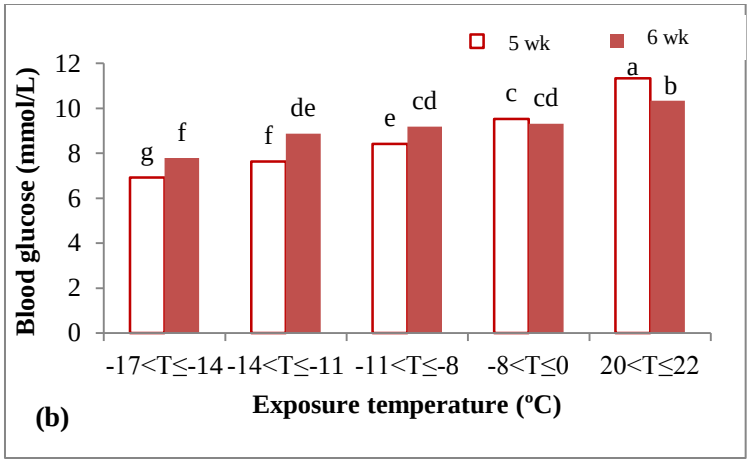


Figure 4-2 Live shrink (a) and blood glucose concentration (b) for 5 and 6 wk birds exposed to different temperatures prior to slaughter.

Table 4-1 Physiological parameters for birds exposed to different temperature, age, lairage and gender groupings.

Variable[1]	Groupings	N	Exp Temp (ºC)	Exp RH (%)	Live shrink (%)	Blood glucose (mmol/L)	CBT during simulated transport (ºC)	CBT during transport and lairage (ºC)	Liver glycogen (µmol/g)
Temperature	20<T≤22	60	21.12±0.7^{a}	22.60±2.6^{c}	1.36±0.8^{c}	10.8±0.7^{a}	40.56±0.3^{a}	40.62±0.3^{a}	23.15±6.2^{a}
	-8<T≤0	76	-3.77±2.7^{b}	60.95±12.3^{a}	3.97±1.2^{ab}	9.41±0.8^{b}	39.87±0.7^{b}	39.77±0.8^{b}	20.23±6.2^{ab}
	-11<T≤-8	70	-9.56±0.9^{c}	50.02±5.3^{b}	3.72±1.1^{b}	8.81±1.6^{c}	38.93±1.3^{c}	38.66±1.8^{c}	20.13±8.8^{ab}
	-14<T≤-11	83	-12.67±0.8^{d}	59.53±7.5^{a}	4.20±1.1^{a}	8.24±1.5^{d}	38.18±2.2^{d}	37.58±3.0^{d}	17.27±4.1^{bc}
	-17<T≤-14	71	-15.15±0.8^{e}	61.21±9.9^{a}	4.03±1.2^{ab}	7.32±1.6^{e}	37.05±2.2^{e}	35.85±4.0^{e}	15.04±3.9^{c}
***P*-value**			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Age	5	180	-4.92	51.79	3.73	8.62	38.24	37.56	18.29
	6	180	-5.17	52.53	3.38	9.09	39.47	39.27	18.7
***P*-value**			**<0.0001**	**NS**	**0.0046**	**0.0121**	**<0.0001**	**<0.0001**	**NS**
Lairage	0	180	-5.04	52.15	3.37	8.78	38.92	38.92	17.04
	2	180	-5.05	52.17	3.74	8.94	38.8	37.92	19.87
***P*-value**			**NS**	**NS**	**0.0006**	**NS**	**NS**	**<0.0001**	**0.0005**
Gender	Female	178	-5.14	52.75	3.48	8.86	39.04	38.68	17.63
	Male	182	-4.95	51.59	3.62	8.86	38.68	38.16	19.58
***P*-value**			**NS**	**NS**	**NS**	**NS**	**0.0121**	**0.0106**	**NS**
Significant Interactions									
Temperature*age			<0.0001	<0.0001	0.0348	<0.0001	<0.0001	<0.0001	0.0004
Temperature*lairage			NS	NS	NS	NS	NS	<0.0001	NS
Lairage*gender			NS	NS	NS	0.0012	NS	NS	0.009

[a-d] Means±SD with different letters are significantly different within a column.

[1]Exp Temp. and RH (temperature and relative humidity in birds' immediate surroundings during 3 h exposure); CBT (core body temperature of birds averaged during 3 h simulated transport; or 3 h of transport and 2 h lairage.

4.5.2 Effect of cold exposure on muscle metabolites

The glycolytic potential (GP), which measures both lactate concentration and potential lactate to be produced from total carbohydrate, measured at 30 h post-mortem showed significantly lower values for breast muscle of birds exposed to extreme cold temperatures (≤ -14ºC) compared to warmer temperature groupings, which were not different from each other (Table 4.2). This shows that at temperatures above -14ºC birds were still able to compensate for cold exposure. But, below this temperature birds were no longer able to compensate for the energy expenditure required to combat the extreme cold temperatures and a significant drop in muscle GP was observed. In addition, no effect of age or lairage was observed on breast muscle metabolites. Lee et al. (1976) did not find any effect of pre-slaughter extreme cold exposure (-20ºC for 6 h) on the glycogen content of *Pectoralis* major muscle of 8 wk broilers at the time of slaughter. Gender showed a significant effect on muscle metabolites, with males having lower lactate and GP values than females (Table 4.2). The lower GP observed for males is coupled with the lower CBT, showing that females are able to better manage their body energy reserve during cold exposure either due to more feather coverage or higher abdominal fat, or simply because females are able to better manage the energy expenditure during severe cold conditions.

4.5.3 Effect of cold exposure on meat quality

Meat quality measures for each temperature classification along with effect of age, lairage, gender and significant interactions between these variables are shown in Tables 4.2 and 4.3. Except for shear values and WBC of breast meat, the interaction between temperature and age was significant for all meat quality parameters, therefore these interactions means are presented in Table 4.4. Breast meat from 5 wk birds was significantly higher in pH_u, darker and redder in color with lower cook loss and higher processing cook yield (PCY), when exposed to temperatures below -8ºC compared to 6 wk old birds and 5 wk old exposed to temperatures warmer than -8ºC (Table 4.4).

Table 4-2 Muscle metabolites and meat quality measures for breast meat of broiler chickens exposed to various temperatures.

Variables[1]	Groupings	N	Lactate (30h) (µmol/g)	GP (30 h) (µmol/g)	pH_u	L*	a*	b*
Temperature	20<T≤22	60	113.18±12.7[a]	121.85±15.1[a]	6.04±0.2[d]	48.76±1.9[a]	2.98±0.9[e]	5.61±1.8[a]
	-8<T≤0	76	118.40±14.5[a]	127.74±15.7[a]	6.04±0.2[d]	47.46±1.8[b]	3.40±0.9[d]	5.18±1.5[a]
	-11<T≤-8	70	118.66±16.6[a]	129.35±18.6[a]	6.17±0.2[c]	46.08±2.7[c]	3.87±1.2[c]	3.86±1.9[b]
	-14<T≤-11	83	113.35±33.5[a]	122.23±34.8[a]	6.30±0.2[b]	45.15±3.3[d]	4.24±1.3[b]	3.44±2.2[b]
	-17<T≤-14	71	93.78±17.0[b]	101.53±18.8[b]	6.36±0.2[a]	44.57±3.7[d]	4.65±1.3[a]	2.78±2.1[c]
***P*-value**			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Age	5	179	107.84	116.62	6.26	45.53	4.34	3.38
	6	180	111.7	120.95	6.12	47.09	3.38	4.87
***P*-value**			**NS**	**NS**	**<0.0001**	**0.0004**	**<0.0001**	**<0.0001**
Lairage	0	180	112.86	122.38	6.15	46.80	3.87	4.33
	2	179	106.75	115.28	6.22	45.83	3.85	3.91
***P*-value**			**NS**	**NS**	**<0.0001**	**0.0049**	**0.169**	**0.0218**
Gender	Female	178	111.67	120.74	6.15	46.62	3.98	4.51
	Male	181	107.57	116.53	6.23	46.01	3.74	3.74
***P*-value**			**0.0379**	**0.036**	**0.0002**	**0.312**	**0.0068**	**0.0042**
Significant Interactions								
Temperature*age			NS	NS	0.0002	0.0095	0.0606	0.0409
Temperature*lairage			NS	NS	0.0154	NS	NS	NS
Age*lairage			NS	NS	0.0749	NS	NS	NS
Temperature*sex			NS	NS	0.0827	NS	NS	NS
Age*lairage*sex			NS	NS	NS	NS	0.0665	0.0089

[a-e] Means with different letters are significantly different within a column. [1]Lactate and GP 30 h (lactate concentration and glycolytic potential measured 30h post-mortem and expressed as µmol of lactate /g of meat); pH_u (ultimate pH measured 30 h post-mortem); L* (lightness), a* (redness), b* (yellowness) of broiler breast meat measured at 30 h post-mortem.

These relationships were less clear-cut for 6 wk birds where an increase in pH_u was observed only when temperature during simulated transport dropped below -11ºC. However, breast meat color of 6 wk birds was darker, redder and less yellow with higher PCY when exposed to temperatures below -14ºC compared to temperatures of -14 to 0ºC, which in turn were significantly different from the control 6 wk birds showing the lightest breast meat with lowest redness, yellowness, WHC and PCY (Table 4.3). The higher pH_u, darker color and higher WBC and PCY was previously reported in Chapter III for birds transported at or below freezing temperatures compared to birds transported at milder temperatures (above 0ºC).

Studies on the effect of cool environmental temperatures prior to slaughter on poultry breast meat quality are limited to those conducted by Babji et al. (1982), Froning et al. (1978), and Holm and Fletcher (1997) and Bianchi et al. (2006) by placing chickens in chamber at 5ºC, immersing turkeys into 4°C ice water for 20 min, placing broilers at environmental chambers set at 7 or 18ºC, or holding birds at temperatures below 12, 12 to 18, or above 18°C respectively. In addition, extreme cold condition was studied by exposing birds to -20ºC for 6 h (Lee et al., 1976). In all of the aforementioned studies exposure to lower temperatures seemed to produce better meat quality characteristics as there was a moderate increase in pH_u of the breast meat that caused slightly darker color and higher water holding capacity for the meat. The increase in pH_u was related to decrease in substrate availability for post-mortem metabolism by Lee et al. (1976) showing lower initial pH and glycogen concentration in the cold-stressed compared to the heat-stressed birds, but no difference with the controls. However, the pH_u values reported by Lee et al. (1976) for controls (5.53±0.03) and extreme cold-stressed (5.52±0.03) birds were much lower than what observed in the current study for controls (6.04±0.2) and those exposed to extreme cold temperatures (<-14ºC) (6.36±0.2).

Table 4-3 Meat quality parameters for birds exposed to different environmental temperatures during simulated transport.

Variables[1]	Groupings	n	Drip loss (%)	Thaw loss (%)	Cook loss (%)	Shear (N/g)	WBC (%)	PCY (%)
Temperature	20<T≤22	60	0.44±0.1^{b}	0.85±0.4^{a}	13.45±1.8^{a}	12.09±1.6^{b}	34.85±8.0^{c}	98.35±8.5^{c}
	-8<T≤0	76	0.38±0.1^{c}	0.59±0.2^{b}	12.44±1.6^{b}	13.06±2.6^{a}	38.23±7.8^{b}	102.11±9.7^{c}
	-11<T≤-8	70	0.41±0.2^{bc}	0.58±0.2^{b}	11.57±2.1^{c}	12.36±1.7^{ab}	43.13±7.8^{a}	108.56±12.9^{b}
	-14<T≤-11	83	0.43±0.1^{b}	0.52±0.2^{b}	10.84±2.1^{d}	12.85±2.0^{a}	45.11±9.2^{a}	113.98±15.9^{a}
	-17<T≤-14	71	0.48±0.15^{a}	0.55±0.32^{b}	10.52±2.3^{d}	13.03±2.6^{a}	43.72±9.6^{a}	115.85±15.3^{a}
***P*-value**			**0.0007**	**<0.0001**	**<0.0001**	**0.0339**	**<0.0001**	**<0.0001**
Age	5	179	0.51	0.65	11.04	12.25	42.22	112.25
	6	180	0.35	0.57	12.35	13.17	40.35	104.10
***P*-value**			**<0.0001**	**0.0005**	**<0.0001**	**0.0266**	**0.0327**	**<0.0001**
Lairage	0	180	0.43	0.63	12.20	12.58	39.60	105.24
	2	179	0.43	0.58	11.19	12.84	42.95	111.07
***P*-value**			**0.5654**	**0.0435**	**<0.0001**	**0.2328**	**0.0008**	**<0.0001**
Gender	Female	178	0.47	0.67	11.94	12.59	39.54	106.52
	Male	181	0.39	0.55	11.46	12.82	42.99	109.78
***P*-value**			**<0.0001**	**0.0162**	**0.2283**	**0.1658**	**0.0015**	**0.0494**
Significant Interactions								
Temperature*age			0.0002	0.0077	0.001	NS	NS	0.0269
Temperature*lairage			NS	NS	NS	NS	0.0223	0.0205
Age*lairage			NS	NS	0.0266	NS	NS	NS
Temperature*gender			0.0074	<0.0001	0.0393	NS	NS	NS

[a-d] Means±SD with different letters are significantly different within a column.

[1]WBC (water binding capacity), and PCY (processing cook yield).

Breast meat of birds exposed to control temperatures showed lower shear values compared to breast meat from birds exposed to simulated transport temperatures below -11ºC (Table 4.3), meaning that exposure to extreme cold temperatures prior to slaughter can cause tougher breast meat. Reports on the effect of cold exposure on tenderness of broiler breast meat are contradictory, where Lee et al. (1976) and Dadgar et al. (2010) did not find any significant effect of cold exposure on tenderness of broiler breast meat, but Babji et al. (1982), and Froning et al. (1978), reported more tender breast meat as a result of moderate cold stress on chicken and turkey breast meat respectively.

It is difficult to discuss the effect of age alone on meat quality in the current study, since significant interactions were observed for age by temperature for the majority of meat quality parameters (Table 4.4). Comparing the 5 and 6 wk birds in the control temperature group did not reveal any differences in pH_u or color lightness of breast meat in the current study (Table 4.4). However, control 5 wk birds showed redder and less yellow breast meat color with higher drip and thaw losses, but lower cook loss and higher PCY compared to 6 wk birds.

It is reported that poultry breast meat tends to become darker and redder as birds age due to increases in myoglobin contents in muscles (Fletcher, 2002), which was not the case in this study. In a more recent study, Bianchi et al. (2006) did not find any differences in redness (a* values) despite a significantly darker color (lower L* values) for heavier birds (> 3.3 kg) compared to the lighter birds (< 3 kg and 3 to 3.3 kg). They associated this darker color to lower post-mortem acidification rather than age differences although neither energy reserve at slaughter nor pH was measured at any time post-mortem. Sandercock et al. (2002) reported higher pH_u, greater drip loss and tenderer breast meat for 35 d old birds compared to 63 d old ones and speculated that the lower pH_u for mature birds was the result of a greater degree of post-mortem glycolytic metabolism in a more mature muscle of the older birds.

Table 4-4 Interaction effect of temperature prior to slaughter and age on breast meat quality parameters.

Age	Groupings	n / group	pH_u[1]	L*	a*	b*	Drip loss (%)	Thaw loss (%)	Cook loss (%)	PCY (%)
5 wk	20<T≤22	30	6.06±0.2^{d}	48.37±1.9^{ab}	3.32±0.8^{d}	5.00±1.7^{bc}	0.48±0.1^{b}	1.00±0.5^{a}	12.95±2.2^{b}	101.80±9.5^{c}
	-8<T≤0	33	6.06±0.2^{d}	47.45±2.3^{bc}	3.67±0.9^{d}	4.81±1.2^{bc}	0.47±0.1^{b}	0.62±0.3^{bc}	12.52±1.9^{bc}	102.88±10.5^{c}
	-11<T≤-8	35	6.28±0.2^{b}	45.14±3.2^{d}	4.45±1.2^{bc}	2.95±1.9^{de}	0.50±0.2^{b}	0.58±0.3^{bc}	10.54±2.2^{ef}	112.98±15.2^{b}
	-14<T≤-11	43	6.40±0.2^{a}	43.70±3.7^{e}	4.91±1.3^{ab}	2.41±2.2^{e}	0.52±0.1^{b}	0.53±0.2^{c}	9.87±2.1^{f}	120.66±17.8^{a}
	-17<T≤-14	38	6.40±0.2^{a}	44.05±4.1^{de}	4.98±1.1^{a}	2.33±2.3^{e}	0.59±0.1^{a}	0.59±0.4^{bc}	10.02±2.5^{f}	118.46±16.9^{ab}
6 wk	20<T≤22	30	6.01±0.1^{d}	49.15±1.7^{a}	2.65±0.9^{e}	6.22±1.8^{a}	0.41±0.1^{c}	0.69±0.2^{b}	13.95±1.2^{a}	94.90±5.6^{d}
	-8<T≤0	43	6.04±0.2^{d}	47.47±1.5^{bc}	3.18±0.8^{d}	5.46±1.7^{ab}	0.32±0.1^{d}	0.58±0.2^{bc}	12.38±1.3^{bc}	101.53±9.1^{c}
	-11<T≤-8	35	6.05±0.1^{d}	47.05±1.6^{bc}	3.28±0.9^{d}	4.80±1.3^{bc}	0.32±0.1^{d}	0.57±0.2^{bc}	12.59±1.3^{bc}	104.14±8.4^{c}
	-14<T≤-11	40	6.18±0.2^{c}	46.71±1.8^{c}	3.53±0.9^{d}	4.54±1.7^{c}	0.34±0.1^{d}	0.51±0.2^{c}	11.89±1.6^{cd}	106.80±9.4^{c}
	-17<T≤-14	32	6.32±0.2^{ab}	45.20±3.1^{d}	4.26±1.5^{c}	3.31±1.9^{d}	0.35±0.1^{d}	0.50±0.3^{c}	11.10±1.9^{de}	112.75±12.9^{b}

[a-d]Means with different letters are significantly different within a column.

[1]pH_u (ultimate pH measured 30 h post-mortem); L* (lightness), a* (redness), b* (yellowness), and PCY (processing cook yield).

Furthermore, significant changes in color of *P. major* muscle of broilers were reported by Anadon (2002) due to age of birds at slaughter, with L* values tending to increase linearly with increase in age for both genders, and b* values increasing, and a* values decreasing with increase in age only for males (Anadon, 2002). A similar trend for both genders was observed for a* and b* values in the current study. Northcutt et al. (1994) found that breast meat of 21 d old broilers had more drip loss than meat from older broilers (28, 35, and 42 d), which was speculated to be a result of alterations in muscle protein isoforms occurring during maturation. Accordingly, higher drip loss was observed for the 5 wk birds compared to the 6 wk birds in the current study, which may be associated with greater surface area for smaller breast samples compared to larger ones. However, the lower cook loss and higher WBC and PCY for 5 wk birds was related to higher breast meat pHu for these birds compared to the 6 wk birds.

The 2 h of lairage following simulated transport resulted in significantly ($P < 0.05$) darker and less yellow (3.9 vs. 4.3) breast meat with lower cook loss and thaw loss compared to meat of birds slaughtered without lairage (Tables 4.2 and 4.3). In addition, there was significant temperature by lairage interaction for breast meat pH_u (Figure 4.3), WBC and PCY (data not shown) with 2 h lairage resulting in higher breast meat pH_u when temperature during simulated transport was below -14ºC and higher WBC and PCY when simulated transport temperatures were below -11ºC, but these properties were not affected by lairage for the control group.

Breast meat from females was redder and yellower with lower pH_u, WBC and PCY, but was not different in L* (lightness) compared to males (Tables 4.2 and 4.3). Furthermore, an interaction effect of temperature by gender was observed on drip, thaw, and cook loss of the breast meat (Figure 4.4) with females showing higher values compared to males when exposed to control and extreme cold conditions ($T \leq -14$ºC) prior to slaughter. However the difference in WHC between genders was not very pronounced when temperature during simulated transport was between 0 and -14ºC (Figure 4.4). All WHC measurement methods followed similar trends, therefore Figure 4.4 only shows the effect for thaw and cook losses. A three way interaction effect of age by lairage by gender was observed on b* with breast meat of 6 wk birds being yellower

compared to the 5 wk birds and females having yellower breast meat compared to males given similar lairage treatments (Data not shown).

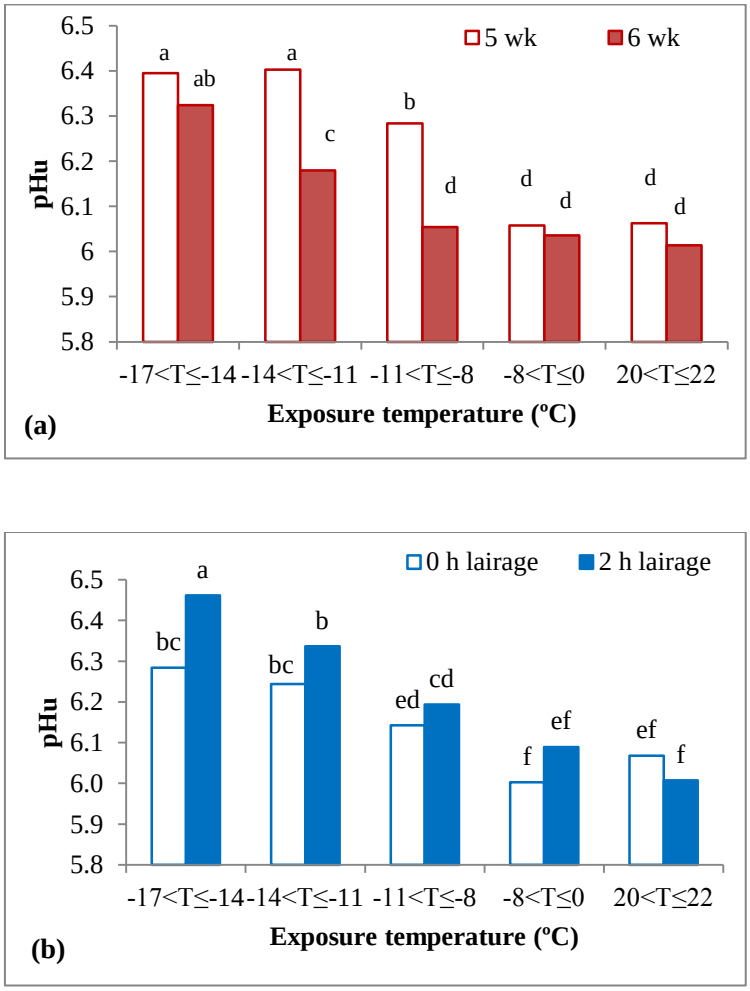


Figure 4-3 Effect of the interaction of temperature by age (a) and temperature by lairage (b) on breast meat ultimate pH.

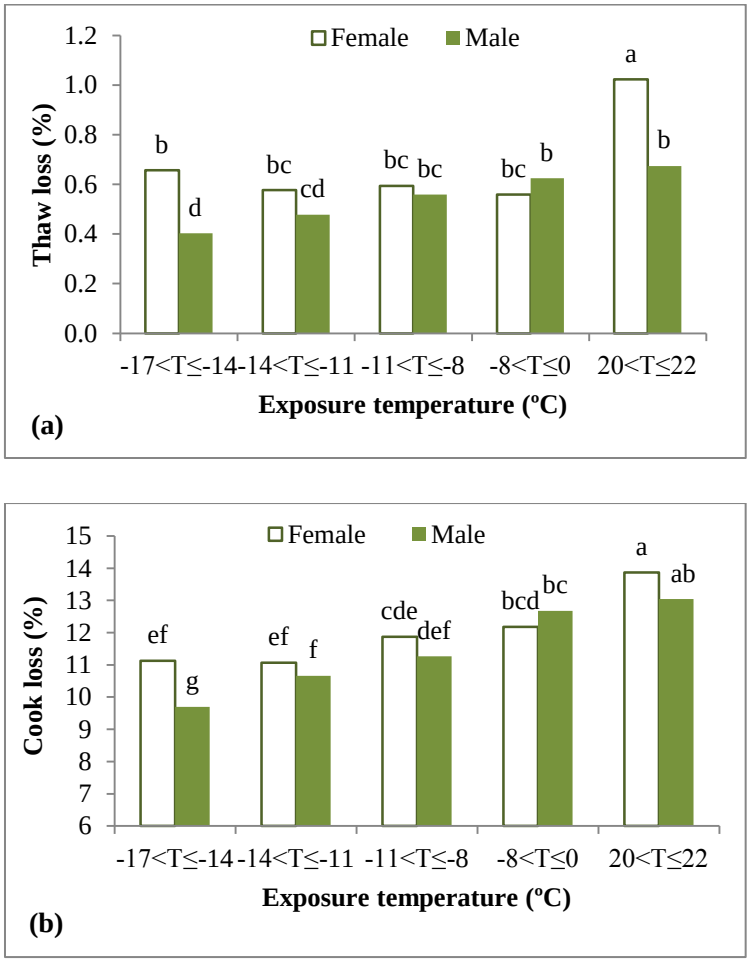


Figure 4-4 Interaction effect of temperature by gender on thaw loss (a) and cook loss (b) of broiler breast meat exposed to different temperature groupings prior to slaughter.

Ngoka et al. (1982) did not observe any effect of gender on breast muscle pH, WHC, cooking loss, or color L*, a* and b* attributes of turkeys. Berri et al. (2007) reported no significant effect of gender on $pH_{u,}$ but a lighter color and higher drip loss for males compared to females that is in contrast to the current study. However, Anadon (2002) reported that *Pectoralis* major muscle of female broilers exhibited lower pH and WHC and higher L*, a* and b* values for females compared to males weighing significantly more than females, which agrees with the current study observations, except for the effect on a* that was not observed here. The higher pH_u observed for males in the current study is most probably related to their lower CBT, since a significant ($P < 0.05$) negative correlation between CBT and pH_u of the breast meat was

observed (Table 4.5). Furthermore, the lower GP reported at 30 h post-mortem supports the higher pH_u for males compared to females. The pH_u correlated well with other meat quality parameters and this explains the darker color and higher water holding ability of males due to higher pH_u compared to females.

4.5.4 Correlations of meat quality parameters with environmental and physiological parameters

Correlations among environmental, physiological and meat quality measures are given in Table 4.5. Environmental conditions (temperature and RH) during the simulated transport showed significant ($P < 0.0001$), but low correlations with bird physiology and meat quality parameters. CBT correlated well with blood glucose, but it could only explain 16-20% of the variation in breast meat pH_u, color and WHC parameters. Glycolytic potential correlated well ($P < 0.0001$) with most meat quality parameters including, pH_u, color L* and b*, cook loss, WBC and PCY, predicting over 50% of the variation in pH_u and therefore being considered as a good measure of meat quality parameters, which agrees with previously reported studies (Soares et al., 2007; Van Laack et al., 2000). The linear relationship between pH_u with lactate and GP was previously reported by Rammouz et al. (2004a) and Berri et al. (2007), but were of a smaller magnitude ($r = -0.47$; $r = -0.57$ respectively). However, since GP measured at 30 h post-mortem could not fully explain the variation in meat quality parameters, other factors than amount of glycogen available at the time of death may have influenced meat quality.

The pH_u was significantly correlated with color L*, a*, b*, thaw loss, cook loss, WBC and PCY (Table 4.5). Several researchers have also demonstrated relationships between pH_u and color (Berri et al., 2007; Rammouz et al., 2004b; Zhang and Barbut, 2005), WBC (Zhang and Barbut, 2005), cook loss (Rammouz et al., 2004 b; Zhang and Barbut, 2005), and drip loss (Berri et al., 2007; Rammouz et al., 2004 b) of poultry breast meat. However, the correlation between the pH_u and drip loss was not significant in the present study, possibly because drip loss was measured following a shorter duration (24 h) than some other reports (Berri et al., 2007; Rammouz et al., 2004 b).

Table 4-5 Pearson correlations between environmental condition, bird physiology and breast meat quality parameters.

Variables[1]	Temp.	RH	pH_u	Lac30 µmol/g	GP30 µmol/g	L*	a*	b*	Thaw loss (%)	Cook loss (%)	WBC (%)	PCY (%)	Live shrink (%)	Blood glucose mmol/L
RH	-0.75**	1.00												
pH_u	-0.41**	0.22**	1.00											
Lac24	0.18*	-0.12	-0.70**	1.00										
GP24	0.17	-0.13	-0.72**	0.99**	1.00									
L*	0.43**	-0.26**	-0.74**	0.51**	0.53**	1.00								
a*	-0.40**	0.24**	0.43**	-0.32**	-0.34**	-0.66**	1.00							
b*	0.40**	-0.22**	-0.73**	0.54**	0.56**	0.82**	-0.59**	1.00						
Thaw loss	0.35**	-0.31**	-0.48**	0.32**	0.34**	0.40**	-0.08	0.36**	1.00					
Cook loss	0.43**	-0.21**	-0.77**	0.51**	0.52**	0.68**	-0.35**	0.65**	0.60**	1.00				
WBC	-0.37**	0.21**	0.79**	-0.47**	-0.48**	-0.61**	0.21**	-0.56**	-0.59**	-0.76**	1.00			
PCY	-0.40**	0.21**	0.90**	-0.62**	-0.63**	-0.73**	0.40**	-0.69**	-0.50**	-0.84**	0.88**	1.00		
Live shrink	-0.63**	0.62**	0.31**	-0.19*	-0.19*	-0.38**	0.34**	-0.36**	-0.40**	-0.42**	0.34**	0.34**	1.00	
Blood glucose	0.62**	-0.36**	-0.55**	0.39**	0.39**	0.49**	-0.43**	0.49**	0.25**	0.43**	-0.37**	-0.50**	-0.35**	1.00
CBT (ºC)	0.52**	-0.23**	-0.46**	0.30**	0.30**	0.39**	-0.42**	0.43**	0.13*	0.37**	-0.22**	-0.37**	-0.21**	0.61**

*, ** significant at $P < 0.05$ and $P < 0.001$ respectively. [1] Temp. (temperature) pH_u (ultimate pH), L* (lightness), a* (redness), b* (yellowness), WBC (water binding capacity), and PCY (processing cook yield).

4.5.5 Effect of cold exposure on incidence of DFD

Birds at both ages showed a very high (~60%) incidence of DFD (dark, firm and dry) breast meat when the exposure temperature was below -14ºC (Figure 4.5). The incidence of DFD breast meat was considerably higher for the 5 wk birds compared to the 6 wk birds at all temperatures tested except for temperatures below -14ºC. In addition, incidence of DFD was up to 20% higher following 2 h lairage (Figure 4.5).

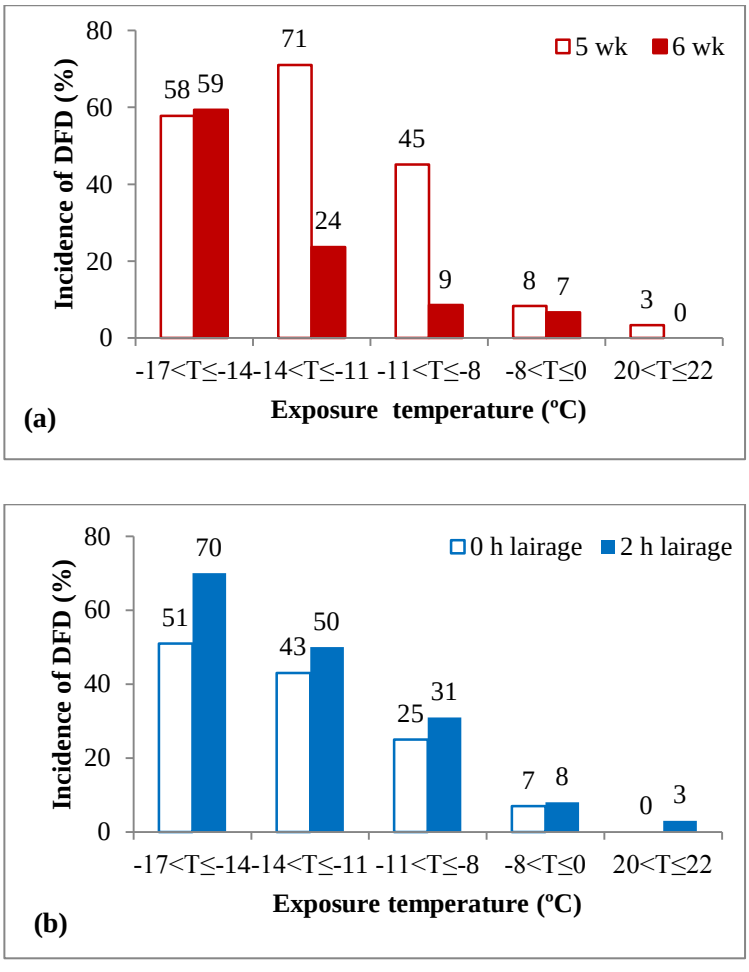


Figure 4-5 Effect of age (5 and 6 weeks) (a) and lairage (b) on incidence of DFD (%) at different temperatures during simulated transport.

Incidence of DFD breast meat has not been extensively studied in poultry. It was shown in Chapter III that cold transportation (below 0 ºC) could increase the incidence of DFD breast meat in broiler chickens (up to 8%). An increase in the incidence of DFD breast meat with seasonal changes from summer to winter was previously reported by Petracci et al. (2004). In addition, 2-10% incidence of DFD has been reported by Lesiow et al. (2007) during the winter season based on the distance from farm to the slaughter plant, with the shortest distance producing the highest incidence of DFD breast meat.

The chamber that was employed in the current study enabled us to simulate temperatures encountered during transportation of broiler chickens, but under more closely controlled conditions. It was interesting to note that there was a similar incidence of DFD reported for temperatures between 0 to -8ºC (7-8%) and also at temperatures between -8 to -11ºC (9%) for the 6 wk old birds (Figure 4.5) to that (8%) observed for 39 to 42 day old birds in Chapter III following exposure to a similar temperature range during transportation. Therefore, this chamber simulated cold transportation reasonably well and is a valuable tool for study of physiological and meat quality parameters in broiler chickens.

The high incidence of DFD at exposure temperatures below -11°C found in the present study has not been reported previously. This may in large part be related to the individual housing of birds during treatment and their inability to huddle together in order to maintain their CBT at acceptable levels. Although commercial broiler transportation during Canadian winters can present exposure to temperatures below -11ºC (Chapter III), it is likely that the ability of birds to huddle together to conserve heat results in a more moderate situation for broilers in transit. Also, since birds transported in a vehicle are experiencing different environmental conditions based on their location inside the truck, only a small proportion of the birds may be exposed to the cold temperatures tested in this study, thus DFD incidence for the whole vehicle would be relatively low.

4.6 Conclusion

Exposure of broiler chickens to extremely cold conditions prior to slaughter resulted in a significant ($P < 0.05$) drop in CBT, increase in live shrink and decrease in blood glucose. Birds at 6 wk of age, which are heavier and have more feather coverage coped better with extreme cold conditions as their meat quality and physiological parameters were significantly compromised when temperature dropped below -14ºC compared to -8ºC for the 5 wk birds, which resulted in breast meat with higher pH_u, darker and redder color, lower cook loss, and higher PCY. Females coped better with cold exposure by maintaining a higher CBT compared to males, which resulted in breast meat with lower pH_u and WHC for females compared to males. GP was only affected when exposure temperature dropped below -14ºC and was shown to be a good predictor of meat quality parameters by explaining over 50% of the variation in pH_u of the muscle, which in turn is highly correlated to other quality parameters of breast meat in broiler chickens. The incidence of DFD breast meat increased to over 50% for the breast meat of birds exposed to cold conditions (T < -11ºC) in individually-sectioned drawers prior to slaughter. In addition, two hours of lairage following extreme cold exposure caused a further increase of 20% in the incidence of DFD breast meat at temperatures below -11ºC. This study shows that it is possible to induce DFD in broilers by cold exposure prior to slaughter, with cold-induced DFD incidence further influenced by age and gender of the birds and length of lairage.

4.7 Connection to the Next Study

As observed in the preceding work, DFD incidence was significantly high when birds were exposed to extreme cold temperatures during simulated transport. It was further shown that cold-induced DFD defect could also be affected by age and gender of birds and lairage duration prior to slaughter. Since, only few studies had looked at DFD breast meat in chickens, it was necessary to further investigate characteristics of this defect in broiler breast meat in relation to muscle energy reserve in order to have a better understanding of the causes of this defect in broiler industry. Therefore in the next study a subset of samples were selected from the previous work to further investigate characteristics of cold-induced DFD breast meat with consideration of different ages at slaughter and different lairage duration prior to slaughter.

5 CHARACTERISTICS OF COLD-INDUCED DARK, FIRM, DRY (DFD) BROILER CHICKENS BREAST MEAT

5.1 Abstract

This study was performed to characterize dark, firm, dry (DFD) breast meat resulting from cold exposure and compare its properties with normal breast meat from cold-exposed and control birds. Effects of liver glycogen concentration at time of slaughter and muscle energy reserves 5 min and 30 h post-mortem on the incidence of DFD breast meat were further investigated. Post-mortem metabolites and quality characteristics of the *Pectoralis* major muscle were evaluated from broilers at 5 and 6 weeks of age exposed to cold temperatures between -18 to -4ºC in a simulated transport system and given 0 or 2 h of rest prior to slaughter. Breast samples were categorized based on ultimate pH (pH_u) and color L* (lightness) values into normal ($5.7 \leq pH_u \leq 6.1$; $46 \leq L^* \leq 53$) breast meat from control (control-normal) or cold-stressed birds (cold-normal) and DFD ($pH_u > 6.1$; $L^* < 46$) breast meat (cold-DFD), which occurred only within the cold-stressed birds. Severity of cold temperatures, age of birds and lairage duration prior to slaughter showed significant effects on the incidence of DFD breast meat with younger birds, colder exposure temperatures and longer rest prior to slaughter causing an increase in the incidence of DFD breast meat. Residual glycogen at time of slaughter was not different between cold-DFD and control-normal breast meat; however, lactate concentration was significantly ($P < 0.05$) lower in cold-DFD compared to control-normal breast meat, but was not different from the cold-normal breast meat. These differences in initial muscle metabolites resulted in lower glycolytic potential (GP) at 5 min post-mortem in DFD breast meat compared to the normal breast meat from both cold-stressed and control birds. However, no difference in initial pH was observed within the cold-stressed birds based on quality defects. Lactate concentration almost tripled for all the samples by 30 h post-mortem that caused a decrease of 0.2-0.3 in pH of normal meat, but did not have any effect on pH of DFD

breast meat. DFD breast meat had significantly higher pH_u (6.4 vs. 6.1), lower L* value (42 vs. 48), lower cook loss (10% vs. 13%), higher water binding capacity (WBC; 49% vs. 39%) and processing cook yield (PCY; 122% vs. 102%) than normal breast meat from both control and cold-stressed birds, which were not different from each other. Therefore, neither glycogen nor lactate concentrations at slaughter explains meat quality defects properly. However, GP could differentiate between normal and DFD breast meat both at slaughter and 30 h post-mortem, yet the stable pH of DFD meat cannot be explained by change in lactate concentration post-mortem.

5.2 Introduction

Dark, firm, dry (DFD) breast meat is a color defect in the meat industry, believed to be related to long-term stress prior to slaughter causing depletion in muscle glycogen, which results in higher post-mortem muscle pH due to prevention of glycolysis by elimination of its substrate. Breast meat with DFD characteristics was investigated in both broiler (Qiao et al., 2001; Barbut et al., 2005) and turkey (Zhang and Barbut, 2005). Meat with this condition is dark in color, has a firm texture and dry appearance or high water-holding capacity (Barbut et al, 2005; Fletcher et al., 2000; Mallia et al., 2000; Rammouz et al., 2004a; Zhang and Barbut, 2005). These characteristics of DFD meat are mainly related to its high pH and higher protein functionality resulting in substantially higher water holding ability, which causes lower light scattering from the surface of meat and subsequently darker color breast meat (Barbut et al., 2005). In addition, the higher water retention of DFD meat contributes to the firm texture and dry surface characteristic of this meat. Furthermore, Barbut et al. (2005) reported that muscle fibers in DFD meat were arranged in a much denser and more compact manner with higher salt soluble protein extraction and higher number of heavy myosin chains compared to the normal meat having fairly loose microstructure with no abnormalities.

Many factors including; transport exhaustion, hunger, fear, climatic stress or aggressive behavior could contribute to the depletion of muscle glycogen and in return limit the amount of lactate formed post-mortem. Darker color of breast meat as a result

of decrease in holding or transport temperature prior to slaughter is previously reported by several authors (Babji et al., 1982; Bianchi et al., 2006; Froning et al., 1978; Holm and Fletcher, 1997). Incidence of DFD in broilers has not been extensively studied; however, Petracci et al. (2004) and Lesiow et al. (2007) reported higher incidence of DFD breast meat during the winter season. In Chapter III of this thesis also a higher incidence of DFD breast meat was reported for birds exposed to temperatures below freezing during transport compared to birds transported under more moderate temperatures.

In post-mortem muscle pH continues to drop due to lactate accumulation until glycogen stores are depleted or metabolic processes stop due to enzymatic arrest caused by low pH (Bendall, 1973). The glycolytic potential (GP) expresses the potential lactate formation in the muscles at exanguination and it is believed that GP at the moment of animal death is capable of predicting the final meat quality (Berri et al., 2005; Hartschuh et al., 2002). The aim of the current study was to investigate the effect of muscle energy reserves (glucose, G6P, glycogen, and lactate) at 5 min and 30 h post slaughter on the incidence of DFD breast meat in broiler chickens and compare its properties to the normal breast meat.

5.3 Materials and Methods

Birds (n=360) at two ages of 5 and 6 wk were placed in a simulated transport system as part of a larger study to investigate transportation temperature on meat quality and animal physiology parameters. Birds were taken off feed 7 h prior to start of the trial, which lead to 10 and 12 h of feed deprivation for the 0 and 2 h rest periods prior to slaughter (lairage) respectively. Birds were wing banded, weighed, and orally dosed with mini temperature loggers (Thermocron iButtons® DS1922L iButtonÂ®, Maxim Integrated Products, CA) into the proventriculus to measure core body temperature (**CBT**) during the trial. The loggers were set to record temperature once each minute and were retrieved after birds were euthanized at the end of the trial. In each trial 30 birds at each age of 5 and 6 wk were exposed to one of the four cold temperatures of -18, -15, -12, and -8 or -4ºC and a control temperature of +20ºC for a duration of 3 h.

Due to the weather changes temperature of -4 could not be attained for the 6 wk birds, therefore these birds were exposed to -8 ºC. The drawers (112cm x 71cm) were partitioned into 15 individual compartments each containing one bird, and placed on top of each other in the chamber. The temperature and humidity of each bird location was monitored once each minute with mini loggers (Hygrocon iButton® DS19223 iButtonÂ®, Maxim Integrated Products, CA). Temperature and RH were interpolated for the center position of each grid space using mapping software (Version 10, Tecplot Inc, Bellevue, WA). At the end of the 3 h exposure the drawers were removed from the chamber. Birds from one of the two drawers, randomly chosen, were weighed and blood samples were collected within 10 min (0 h lairage); birds from the other drawer were allowed to remain in the mesh covered drawer at room temperature for 2 h prior to weighing and blood sampling. Live shrink was calculated as the difference between the live weights measured before and after the 3-h treatment. Blood glucose levels were measured using a glucose kit (i-STAT^® 1 Handheld Clinical Analyzer, Heska Inc., Loveland, CO).

5.3.1 Meat quality measurements

Birds were hand slaughtered in a simulated commercial abattoir as described in Chapter IV. Immediately after defeathering, core samples were collected from the upper part of the right breast muscle (*Pectoralis* major), at 3-5 min post-mortem, frozen in liquid nitrogen and stored at -80ºC. These samples were subsequently used to establish muscle initial pH and glycolytic potential (GP). Liver samples were collected immediately following evisceration (5-8 min post-mortem) from the smallest lobe of the liver, frozen in liquid nitrogen and stored at -80ºC. Carcasses were placed into a 0°C chill tank 20 min post-mortem for 40 min, with frequent agitation.

Chickens were deboned at 6-7 h post-mortem. Breast meat was then placed on styrofoam trays and stored in a 4ºC cooler for further analysis. Color L*, a* and b* were measured on breast meat (*Pectoralis* major) at 30 h post-mortem using Minolta Chroma meter RC-400 according to Dadgar et al. (2010). Following analysis, breast meat pieces were vacuum-packed individually and frozen at -30ºC for further analyses.

Thaw loss (% weight lost during thawing overnight at 4ºC), cook loss (% weight lost during cooking to internal temperature of 75ºC), and Warner Bratzler shear force (average shear force over 10 samples cut parallel to muscle fiber with 1 cm^2 cross section from each cooked breast meat) were measured on intact breast meat and water binding capacity (WBC), processing cook yield (PCY) and ultimate pH (pH_u) (using slurry method) were determined on each ground breast fillet according to Dadgar et al. (2010).

Glycogen (muscle and liver), glucose/G6P (muscle) concentrations were determined using the method modified from Passonneau and Lauderdale (1974). A Glucose Hexokinase assay kit (Sigma, GAHK-20) was used to measure the concentration of the generated NADPH, which was equivalent to total glucose, using a spectrophotometer at 340 nm. Total carbohydrate content was expressed in μmol glucosyl units per gram weight of the sample. Lactate concentration was measured according to Hartschuh et al. (2002) as described in chapter IV, and GP was calculated based on the equation proposed by Monin and Sellier (1985):

$$GP = 2 \times (glucose + G6P + glycogen) + lactate$$

5.3.2 Classification of samples into quality groups

From each of the 6 temperature treatments (5 cold treatments of -18 to -4ºC, and a control of +20ºC), 10 birds (5 birds with 0 h and 5 birds with 2 h lairage) were selected from each age (5 and 6 wk old) from the first row of the simulated transport system to measure post-mortem muscle metabolites and breast meat quality (total of 120 samples). However, later another 20 samples were tested to reaffirm some of the results observed in GP measurement. Therefore, a total of 140 birds were tested. Breast meat samples were categorized into dark, firm, dry (DFD) (pH> 6.1 and L*< 46.0) breast meat, generated from cold-stressed birds, and normal (5.7≤pH≤6.1 and 46.0≤L*≤53) breast meat from either cold-stressed or control birds. These groupings were adopted from Barbut et al. (2005). No sample with pale, soft, and exudative (PSE) (pH<5.7 and L*>53.0) characteristics was observed among the breast meat samples evaluated.

5.3.3 Statistical analysis

A completely randomized design with a 3 x 2 x 2 x 2 factorial arrangement was employed in this experiment, with effect of quality status (control-normal, control-DFD and cold-DFD) as the main source of variation in the model and effect of age (5 or 6 wk), lairage (0 or 2 h) and gender of the birds as sub factors with variable samples per group. Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (SAS Institute, Cary, NC) and results were reported as least square means with their standard deviations. Differences among means were evaluated using the Duncan's multiple comparison test option of SAS (SAS Institute, Cary, NC). Unless otherwise stated, the means were considered different at a $P \leq 0.05$ by ANOVA. Pearson's correlation coefficients (r), regression model (R^2), and probabilities were generated using the correlation procedures of SAS.

5.4 Results and Discussion

5.4.1 Bird physiology parameters and occurrence of DFD breast meat

Birds were grouped based on meat quality defects into three groups of normal from cold-stressed or control birds and DFD breast meat from cold-stressed birds. In the larger study that these samples were sourced from, exposure of birds to colder temperatures prior to slaughter increased the incidence of DFD breast meat (Chapter IV). Also, as shown in Table 5.1, the average temperature this subset of birds experienced was significantly lower for cold-stressed birds with DFD defect (-13.6ºC) compared to their normal counterparts (-9.3ºC). In addition, birds with DFD breast meat showed significantly ($P < 0.05$) lower core body temperature (CBT) compared to their normal counterparts (Table 5.1). The control birds, experiencing temperatures of 21ºC during treatment showed the highest CBT of all. In addition, blood glucose levels were significantly lower for cold-stressed birds with DFD breast meat compared to the cold-stressed birds with normal breast meat, showing that blood glucose levels below 8 mmol/L was associated with development of DFD breast meat. However, blood glucose

level was also significantly lower in normal breast meat of cold-stressed birds compared to the control birds (Table 5.1).

Table 5-1 Bird physiology parameters based on quality groupings (n = 139).

Variables [1]		N	Exp Temp (ºC)	CBT (ºC)	Live shrink (%)	Blood glucose (mmol/L)	Liver glycogen (μmol/g)
Control		19	20.62±0.3[a]	40.58±0.3[a]	1.54±1.0[b]	10.75±0.7[a]	23.03±6.5[a]
Cold-Normal		67	-9.28±5.7[b]	38.84±2.0[b]	3.75±1.2[a]	8.94±1.3[b]	19.75±6.7[b]
Cold-DFD		53	-13.58±2.6[c]	37.61±1.6[c]	4.30±1.1[a]	7.36±1.6[c]	15.14±4.0[c]
***P*-value**			**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Age (wk)	5	69	-7.08	38.07	3.90a	8.32	18.15
	6	70	-6.98	39.11	3.44b	8.80	18.70
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**
Lairage (h)	0	70	-6.75	38.84	3.45b	8.51	17.04b
	2	69	-7.31	38.35	3.89a	8.62	19.76a
***P*-value**			**NS**	**NS**	**NS**	**NS**	**0.01**
Sex	F	76	-9.21b	38.62	3.80	8.29	17.63
	M	63	-4.43a	38.56	3.51	8.90	19.44
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**

[a-d] Means±SD with different letters are significantly different within a column.

[1]Exp Temp (temperature in birds' immediate surroundings during 3 h exposure); CBT (core body temperature of birds averaged during 3 h simulated transport).

Liver glycogen was substantially lower in cold-stressed birds with DFD breast meat compared to cold-stressed birds with normal breast meat, which in turn was significantly lower than the controls (Table 5.1). Therefore, the low level of glycogen in liver could be an early indicator of DFD breast meat. No significant difference was observed in physiology parameters based on age, lairage or gender of the birds (Table 5.1) except for liver glycogen. Birds with 2 h of rest prior to slaughter showed significantly ($P<0.05$) higher liver glycogen content (17.0 vs. 19.8 μmol/g) compared to the birds slaughtered immediately after slaughter, which might be related to the ability of birds to recover or rebuild their liver glycogen reserve from other sources.

5.4.2 Muscle metabolites and occurrence of DFD breast meat

The glycogen reserve at time of slaughter was not different between DFD and normal breast meat of the control birds, but was lower compared to the normal breast meat of the cold-stressed birds (Table 5.2). The lactate concentration was significantly lower at 5 min post-mortem for cold-stressed birds regardless of the quality defect compared to the control birds. No difference in glucose/G6P was observed based on quality groupings. However, glycolytic potential, that is the potential of the muscle to produce lactate, calculated based on glucose equivalents and lactate at 5 min post-mortem was significantly lower for breast meat of cold-stressed birds with DFD defect, compared to normal breast meat from either cold-stressed or control birds which were not different from each other.

The glycogen reserve was almost completely used up by 30 h post-mortem in all breast meat samples regardless of quality grouping. Glucose/G6P concentration at 30 h post-mortem was not different between DFD and normal breast meat of the control birds, but was higher in normal breast meat of the cold-stressed birds, which might be related to the difference in initial glycogen between the two groups. Lactate concentration and GP at 30 h post-mortem were significantly lower for DFD breast meat compared to normal breast meat of both cold-stressed and control birds, which were not different from each other. The lower lactate concentration and GP at 30 h post-mortem could explain the higher pH of the DFD compared to normal breast meat, but there was a threefold increase in lactate concentration for all the samples yet no change in pH of the DFD breast meat.

According to Puolanne et al. (2002), 10-20 mmol lactic acid per kg of meat, derived from 5-10 mmol of glucose equivalents is required to make a change of 0.2-0.3 units in pH. Therefore, one would expect some drop in pH of DFD meat with ~65 μmol/g build up in lactate concentration and perhaps more drop in pH of normal meat with 68 and 83 μmol/g increases in lactate concentration of normal breast meat of control and cold-stressed birds respectively. Consequently, neither initial glycogen nor initial lactate alone was able to differentiate the ultimate quality of broiler breast meat,

Table 5-2 Breast muscle metabolites at 5 min and 30 h post-mortem for different quality groupings (n = 139).

Variables[1]		n	pH_i	Glucose /G6P	Lactate 5min	Glycogen 5 min	GP 5 min	n	Glucose /G6P	Lactate 30h	Glycogen 30 h	GP 30 h
Control		19	6.31±0.1[b]	3.4±1.3	44.9±14.6[a]	30.0±7.9[b]	105.7±15.0[a]	19	1.6±0.7[b]	113.5±11.3[a]	3.0±1.9	121.9±13.7[a]
Cold-normal		61[2]	6.42±0.1[a]	3.7±1.6	36.8±10.9[b]	36.1±10.2[a]	108.9±20.5[a]	67	2.6±1.9[a]	119.7±22.4[a]	3.0±2.4	130.2±23.5[a]
Cold-DFD		39[2]	6.46±0.2[a]	2.8±1.7	31.1±14.3[b]	26.4±7.9[b]	84.0±22.5[b]	53	1.1±1.4[b]	96.4±19.3[b]	2.8±1.7	103.7±20.5[b]
***P*-value**			**0.00**	**NS**	**0.02**	**0.01**	**0.082**		**0.0002**	**<0.0001**	**NS**	**<0.0001**
Age (wk)	5	69	6.45	2.85	34.27	29.81	93.46	69	1.32	108.11	3.42	116.91
	6	50	6.38	4.02	38.70	34.70	108.97	70	2.49	111.70	2.44	120.95
***P*-value**			**0.044**	**0.002**	**NS**	**NS**	**NS**		**0.022**	**0.006**	**0.01**	**NS**
Lairage (h)	0	60	6.45	3.65	37.34	32.10	102.22	70	2.27a	112.86	2.82	122.38
	2	59	6.38	3.05	34.97	31.69	97.91	69	1.56b	107.00	3.02	115.54
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**		**NS**	**NS**	**NS**	**NS**
Sex	F	62	6.45	3.23	35.80	32.31	100.22	76	1.90	111.67	2.94	120.74
	M	57	6.38	3.49	36.56	31.45	99.94	63	1.94	107.86	2.90	116.84
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**		**NS**	**NS**	**NS**	**NS**
Significant Correlations												
Group* age			NS	0.0003	0.004	NS	0.039		NS	NS	NS	NS
Group*lairage			NS	NS	NS	NS	NS		0.039	NS	NS	NS
Age*lairage			NS	NS	NS	NS	NS		NS	NS	NS	0.049
Lairage*gender			NS	NS	0.036	NS	NS		NS	NS	NS	NS
Group*lairage*gender			NS	NS	NS	NS	NS		NS	0.049	0.049	NS

[a-d] Means±SD with different letters are significantly different within a column.

[1] pH_i (initial pH measured at 5 min post-mortem); Glucose/G6P, Lactate, Glycogen (glucose or G6P, lactate and glycogen concentrations (μmol/g) measured at 5 min or 30 h post-mortem); GP (glycolytic potential calculated based on metabolites at 5 min or 30 h post-mortem).[2] Twenty samples collected at 5 min were lost from 6 wk birds exposed to treatment temperature of -18.

whereas, GP measured at either 5 min or 30 h post-mortem was able to predict quality attributes of broiler chickens breast meat, yet was not able to explain the lactate build up of the DFD breast meat.

No significant differences were observed based on age, lairage or gender on initial muscle metabolites. Significant interaction effect of quality groupings and age was observed for initial glucose/G6P, lactate and GP, with DFD breast meat of 6 wk birds showing higher Glucose/G6P, lactate and GP compared to the DFD breast meat of 5 wk birds (Figure 5.1). However, breast meat of the 6 wk control birds had lower Glucose/G6P and lactate compared to the 5 wk controls. This age effect on initial muscle metabolites might be related to the differences in ability of birds to cope with cold exposure prior to slaughter at different ages (Chapter IV), with 6 wk birds being able to better survive the extreme cold temperatures. It should be noted that some of the samples for initial metabolites measurement were lost from the 6 wk birds exposed to treatment temperatures of -18, resulting in an imbalance of samples between ages.

A significant interaction effect of lairage by gender was observed for initial lactate concentration (Figure 5.2), with males showing higher lactate concentration at 5 min post-mortem, when slaughtered immediately after slaughter compared to males slaughtered after 2 h, but no difference was observed between the genders regardless of lairage duration. Moreover, the 2 h lairage seemed to substantially decrease the ultimate Glucose/G6P of the normal breast meat from the cold-stressed birds (Figure 5.2) and substantially decrease the GP of the 5 wk birds (Figure 5.3), which might be explained by usage of more energy by cold-stressed birds to recover their CBT during the 2 h rest prior to slaughter. In addition, due to the higher drop in CBT of the 5 wk birds during cold exposure they would have needed more energy to recover to their normal CBT during the rest period prior to slaughter (Chapter IV).

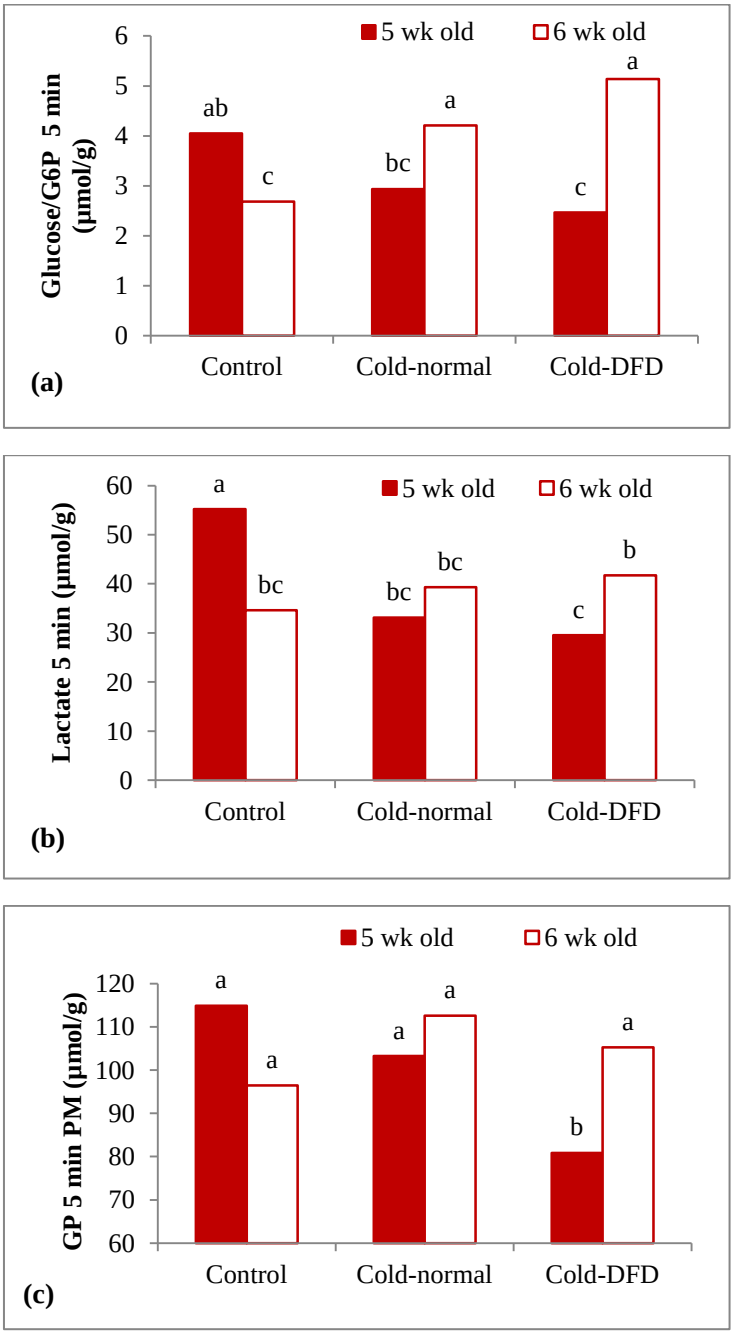


Figure 5-1 Interaction effect of quality groupings and age of the bird at slaughter on Glucose/G6P (a), lactate (b) and glycolytic potential (c) measured at 5 min post-mortem.

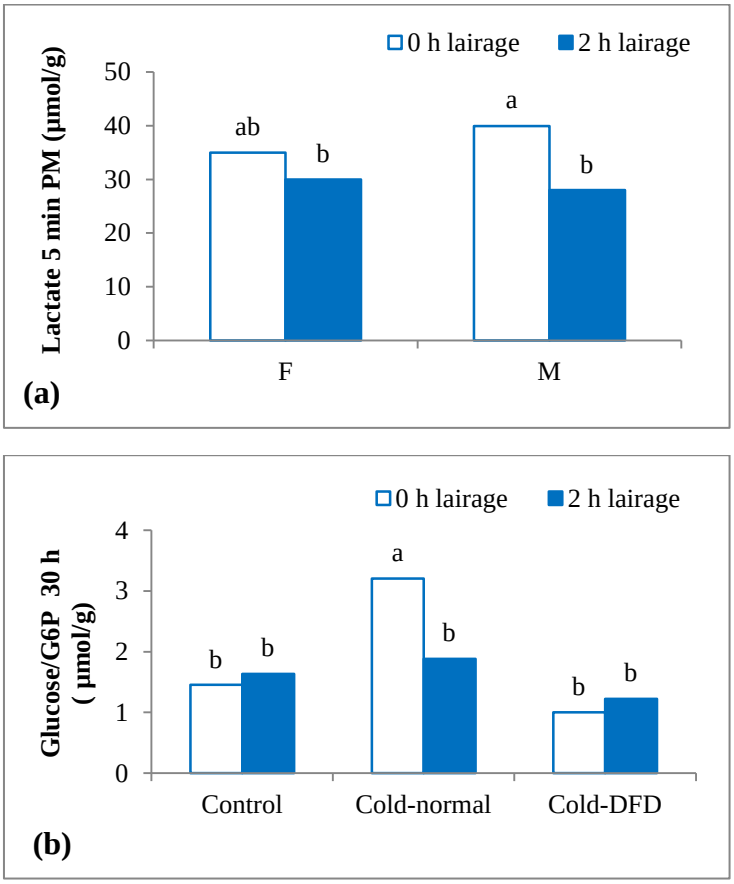


Figure 5-2 Interaction effect of lairage by gender on lactate concentration at 5 min post-mortem (a) and quality grouping by lairage on Glucose/G6P measured at 30 h post-mortem (b).

Development of DFD breast meat in the presence of available energy reserves at time of slaughter was not expected. Under cold exposure, the DFD breast meat had the potential to develop meat with normal properties, but for some reason the produced lactate from the glycogen did not contribute to a drop in pH. Therefore, glycogen reserve at slaughter may not be the only reason for the incidence of DFD breast meat. Bendall (1973) have previously demonstrated that pH decline can stop, even in the presence of high residual glycogen content, although the reasons for this phenomenon are not clearly understood. According to Scopes (1971), inactivation of glycogenolytic and glycolytic enzymes resulting from disappearance of AMP due to deamination of AMP into IMP could explain the termination of pH decline, even in the presence of glycogen.

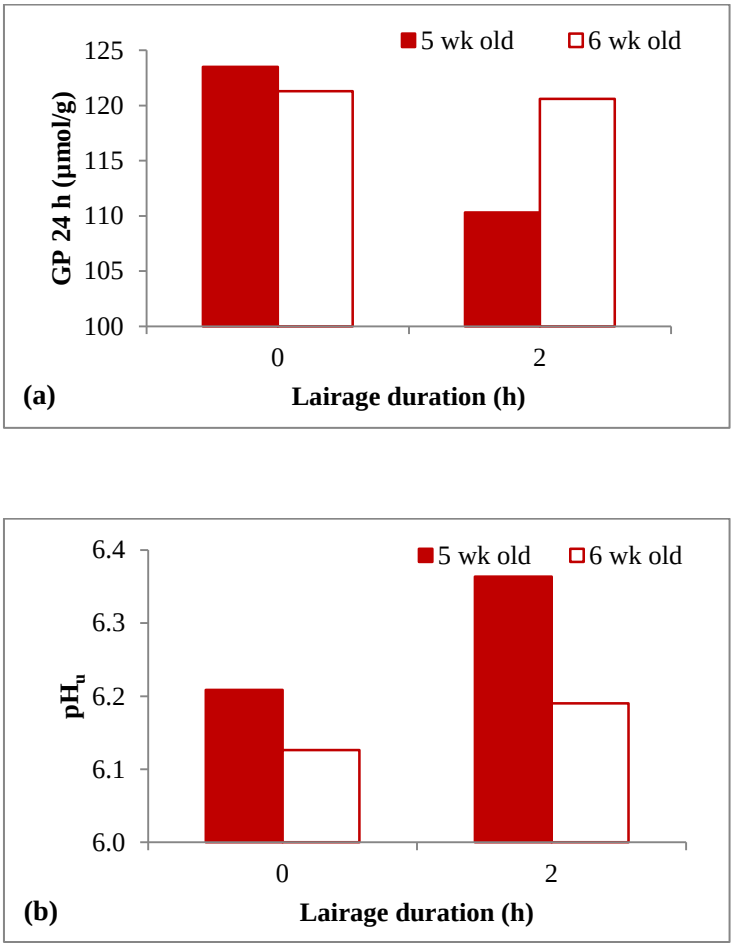


Figure 5-3 Interaction effect of age and lairage on glycolytic potential (a) and pH_u of breast meat (b).

Concentration of glycogen in the liver was observed to be lower compared to breast muscle immediately after slaughter regardless of meat defect or lairage duration. This finding was reported earlier for young chicks (4 weeks old) in the study by Golden and Long (1942 a, b). These researchers reported a decline of 24 mg in liver glycogen (28 to 4 mg) compared to only 3 mg in muscle glycogen (11 to 8 mg) after a 24 h fast.

A substantial difference (~20 µmol/g) was observed between the GP measured at 5 min post-mortem compared to GP measured at 30 h post-mortem. The higher GP calculated at 30 h post-mortem was most probably related to the high lactate concentration measured at 30 h post-mortem as the ultimate Glucose/G6P and glycogen were decreased to negligible values by 30 h post-mortem and would not contribute

much to the GP calculation. The difference observed between GP measured at 5 min and 30 h post-mortem was not expected, since the GP calculation is supposedly bringing into account major glycolysis by-products and is not time dependent. Therefore, one speculation is that either we did not measure all the glycogen at 5 min post-mortem or the glycogen was in a form that could not be measured by this method. Maribo et al. (1999) reported a high correlation (0.83) between the 4 min and 30 h GP for the *longissimus dorsi* muscle of pigs compared to the lower correlation of 0.55 observed in this study for breast muscle of chickens. This discrepancy between the porcine *longissimus dorsi* muscle and chickens *Pectoralis* major muscle might be more muscle type or fiber type related, which needs further investigation. Concentration of glycogen and Glucose/G6P were higher and that of lactate was lower immediately after exanguination compared to sampling the day after slaughter. No change in the concentration of G6P was reported with sampling time by Maribo et al. (1999). The GP values reported for normal breast meat of control and cold-stressed birds in this study was similar to initial GP values reported for breast meat of broilers exposed to minimal stress prior to slaughter (107μmol/g) by Rammouz et al. (2004b).

5.4.4 Quality characteristics and incidence of DFD breast meat

Breast meat with DFD defect had significantly ($P < 0.0001$) higher pH_u (by 0.4 units), was darker (42 vs. 48), redder (~1.5 unit redder) and less yellow (by 3 units) compared to normal meat (Table 5.3). In addition, a higher water holding capacity for both intact and ground meat was observed for DFD breast meat, as evidenced by the significantly ($P < 0.01$) lower thaw and cook loss and higher water binding capacity (WBC) and processing cook yield (PCY). These characteristics of DFD meat are in agreement with previously reported studies (Allen et al., 1998; Barbut et al., 2005; Mallia et al., 2000; Zhang and Barbut, 2005).

Table 5-3 Meat quality characteristics for DFD and normal breast meat of cold-exposed and control birds (n = 139).

Variables [1]		n	pH_u	L*	a*	b*	Thaw loss (%)	Cook loss (%)	Shear force (N/g)	WBC (%)	PCY (%)
Control		19	6.09±0.1[b]	48.3±1.7[a]	3 .0±1.1[c]	5.3±1.6[a]	0.79±0.4[a]	13.5±1.9[a]	12.0±1.1	38.4±5.3[b]	99.5±6.6[b]
Cold-normal		67	6.08±0.2[b]	47.8±1.7[a]	3.6±1.1[b]	5.0±1.4[a]	0.61±0.3[b]	12.5±1.6[b]	13.0±2.2	39.2±8.4[b]	103.0±9.2[b]
Cold-DFD		53	6.44±0.2[a]	42.4±2.6[b]	4.8±1.2[a]	1.9±1.5[b]	0.44±0.2[c]	9.8±2.0[c]	12.4±1.7	49.2±7.0[a]	122.6±12.7[a]
***P*-value**			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**NS**	**<0.0001**	**<0.0001**
Age (wk)	5	69	6.29a	45.05	4.54	3.18	0.62	11.03	12.22	43.20	113.19a
	6	70	6.16b	46.52	3.50	4.50	0.52	12.06	13.08	42.67	107.02b
***P*-value**			**0.059**	**NS**	**0.04**	**0.03**	**0.015**	**0.035**	**NS**	**NS**	**NS**
Lairage (h)	0	70	6.17	46.37	4.05	4.20	0.63a	12.29a	12.51	40.09b	105.57b
	2	69	6.28	45.20	3.98	3.48	0.52b	10.82b	12.80	45.77a	114.54a
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**	**0.001**	**NS**	**0.017**	**0.013**
Sex	F	76	6.21	45.82	4.26	3.95	0.59	11.53	12.55	42.34	110.29
	M	63	6.23	45.75	3.73	3.72	0.55	11.58	12.78	43.63	109.78
***P*-value**			**NS**	**NS**	**NS**	**NS**	**0.028**	**NS**	**NS**	**NS**	**NS**
Significant Interactions											
Group* age			NS	NS	NS	NS	NS	NS	NS	0.038	NS
Age*lairage			0.034	NS	NS	NS	NS	NS	NS	NS	NS
Age*gender			NS	NS	NS	0.022	NS	NS	NS	NS	NS
Group*age*gender			NS	NS	NS	0.003	NS	NS	NS	NS	NS
Age*lairage*gender			NS	0.025	NS	NS	NS	NS	NS	NS	NS

[a-d] Means±SD with different letters are significantly different within a column.

[1] pH_u (ultimate pH), L*, a*, b* (color lightness, redness and yellowness), WBC (water binding capacity), PCY (processing cook yield).

Breast meat from cold-stressed birds had significantly higher initial pH (pH_i measured at 5 min post-mortem) compared to the control birds, but by 30 h post-mortem the pH of DFD birds remained the same, but that of normal breast meat from the cold-stressed birds decreased to values similar to pH of breast meat from the control birds. Therefore, it can be speculated that WHC (measured by evaluating thaw and cook loss) was more related to initial pH or rate of drop in pH, whereas WBC and PCY are mainly pH_u dependent. It is also interesting to note that normal meat lightness and yellowness are not different between the controls and cold-stressed birds, whereas a* values are already shifted to higher values due to cold exposure, yet it is lower than those of DFD breast meat. Therefore, it can be said that L* and b* are more pH_u dependent, whereas, a* value might be affected by other factors such as rate of drop in pH other than pH_u. It might be beneficial to monitor pH drop over time to have more understanding of how it affects breast meat quality properties. No difference in shear value was reported based on quality groupings, which is in agreement with previously reported studies (Dadgar et al., 2010; Lee et al., 1976).

Age of birds showed a significant effect on initial pH, color a*, b*, thaw loss, and cook loss of the breast meat, with 6 wk birds showing lower initial pH, less red, but yellower breast meat color, lower thaw loss and higher cook loss compared to breast meat of 5 wk birds (Table 5.3). Significant interaction of age by lairage was observed on pH_u (Figure 5.3), with 6 wk birds showing significantly lower pH_u when given 2 h rest prior to slaughter compared to 5 wk birds. The 2 h lairage resulted in an overall higher pH_u for both ages. In addition, significant interaction effect of quality groupings by age was observed on WBC (Figure 5.4), breast meat of both 5 and 6 wk birds with DFD characteristics showed higher WBC compared to the normal quality breast meat. Quality grouping by age and gender interactions were significant for color b* (Figure 5.4), where breast meat with DFD defect and normal breast meat of controls from 6 wk females showed yellower breast meat compared to 5 wk females and 6 wk males, but no significant difference was observed on normal breast meat b* value of cold-stressed broilers based on age or gender. In addition, age by lairage by gender showed a significant effect on color L* (Data not shown).

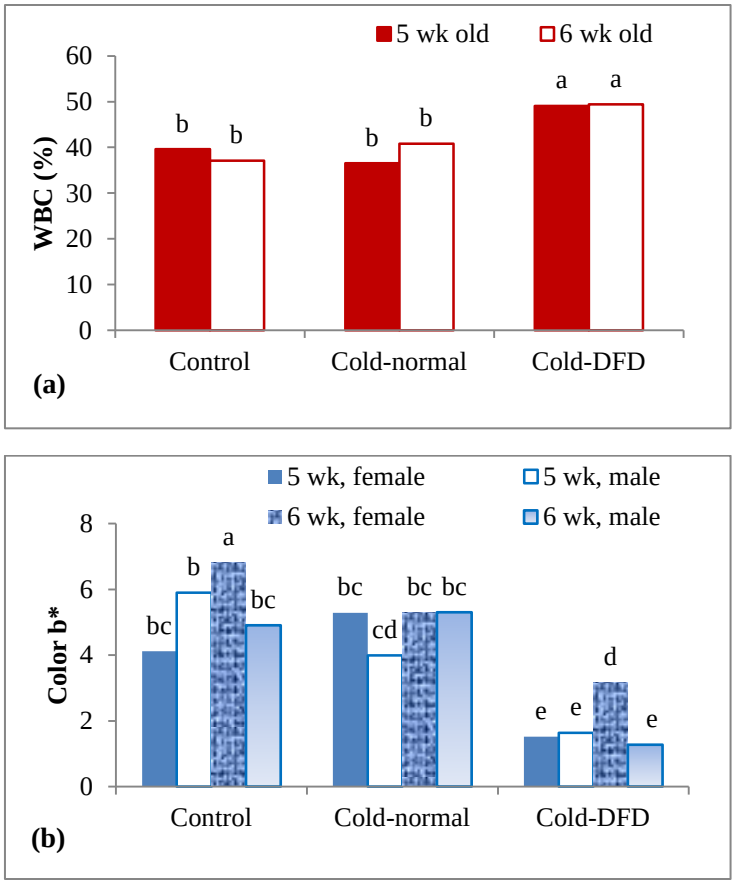


Figure 5-4 Interaction effect of quality grouping and age on water binding capacity (a), and quality grouping, age and gender on color b* (b).

Incidence of DFD breast meat was higher for 5 wk birds compared to 6 wk birds regardless of lairage or gender (Figure 5.5). The 2 h lairage following cold exposure increased the incidence of DFD breast meat, particularly in 5 wk old females and 6 wk old males (Figure 5.5). Six wk birds slaughtered immediately following treatment showed the lowest incidence of DFD breast meat. The high incidence of DFD breast meat at average simulated transport temperatures of -13.6ºC was explained by increased utilization of muscle and liver energy reserves in order for birds to maintain their shivering thermogenesis while exposed to the cold environmental conditions. However, glycogen reserve at slaughter was not different based on quality grouping. But, liver glycogen was significantly lower for birds with DFD breast meat, as was blood glucose.

Furthermore, GP that is the potential of muscle to produce lactate and bring down the pH was significantly lower in breast meat of birds with DFD defect.

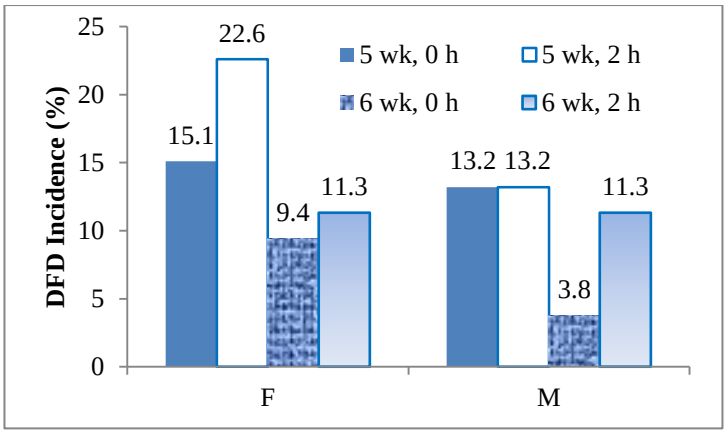


Figure 5-5 Incidence of DFD breast meat in broiler chickens based on age of the birds (5 or 6 wk old), lairage duration (0 or 2 h) and gender at slaughter.

5.4.3 Correlations between muscle metabolites and meat quality parameters

The pH_u of breast meat in the current study was highly correlated to GP measured at 5 min (r = -0.70) and 30 h (r = -0.72) post-mortem (Table 5.4). Similarly, Soares et al. (2007), and Van Laack et al. (2000) reported high correlation of -0.70 between pH_u and GP values. However, other studies have reported lower correlations between GP and pH_u of LD muscle of pigs (r = 0.61) (Maribo et al., 1999), and breast muscle of turkeys (r = -0.44) (Rammouz et al., 2004a) and broilers (r = -0.36 and r = -0.42) (Rammouz et al., 2004b; Berri et al., 2005). Initial muscle glycogen concentration was poorly correlated to meat quality parameters in the current study which is in accordance with previously reported studies (Rammouz et al., 2004 a, b).

GP values reported in the current study could predict 50% of the variation in pH_u, 40% of the variation in processing PCY and 25% of the variation in color L*, b*, cook loss and WBC of the breast meat regardless of the time of testing (5 min or 30 h post-mortem). Increased GP was associated with lower pH_u and higher L*, WBC and PCY in this study. However, it was previously reported that GP could predict 20% of the variation in pH_u when only normal breast meat was evaluated (Rammouz et al.,

2004b). Berri et al. (2005) reported positive significant ($P < 0.01$) correlations between GP and initial pH (15 min; r= 0.31), and L* (r = 0.27) of breast meat from fast growing line of broilers. Whereas, the correlations between initial GP with pH_i (-0.27) was negative in the current study (Table 5.4). The pH_i did not show high correlation (r < 0.39) with any of meat quality parameters, showing that it is not a valuable parameter for predicting final meat quality attributes. Similarly, previous studies have reported that pH_u and ultimate quality of the meat are not influenced by early post-mortem pH (Anadon, 2002; Berri et al., 2001). On the other hand, some studies have found high correlations between initial pH and meat quality attributes and suggested that testing immediately after slaughter can give a good indication of meat color and pH after aging (Mallia et al., 2000).

Table 5-4 Correlations between biochemical properties and meat quality traits.

	Glycogen 5 min	GP 5 min	Lactate 30 h	GP 30h	pH_u	L*	WBC	PCY
Glycogen 5min	1.00							
GP5min	0.79**	1.00						
Lactate 30h	0.48*	0.54*	1.00					
GP30h	0.50*	0.55*	0.99*	1.00				
pHu	-0.56*	-0.70**	-0.70**	-0.72**	1.00			
L*	0.34*	0.51*	0.51*	0.53*	-0.78**	1.00		
WBC	-0.38*	-0.43*	-0.48*	-0.49*	0.76*	-0.60*	1.00	
PCY	-0.45*	-0.56*	-0.62*	-0.63*	0.91*	-0.74*	0.87*	1.00

*, ** significant at $P < 0.05$ and $P < 0.001$ respectively.

[1]Glycogen 5 min (glycogen reserve measured at 5 min post-mortem); GP (glycolytic potential measured at 5 min or 30 h post-mortem); Lactate 30 h (lactate concentration measured at 30 h post-mortem; pH_u (ultimate pH); L* (color lightness); WBC (water binding capacity); PCY (processing cook yield).

The high correlation (99%) between GP and lactate at 30 h post-mortem enabled lactate concentration to predict the ultimate quality characteristics of the breast meat to a similar extent as GP. However, neither could explain the lactate build up in the DFD breast meat despite a lack of change in pH. The high correlation reported between lactate concentration and breast pH_u in this study was not supported by previous studies of fast growing broiler chickens (Rammouz et al., 2004b; VanLaack et al., 2000). The pH_u was highly correlated with all meat quality parameters (Table 5.4), showing the importance of this factor on meat quality attributes. Finally GP could only predict about 50% of the variation in pH_u and ultimate meat quality parameters. This opens the path for considering other important factors that might influence post-mortem metabolism and subsequent broiler breast meat quality such as enzymes involved in glycogenolysis and glycolysis, which needs further investigation.

5.5 Conclusion

DFD breast meat is a defect for the fresh meat industry; however, it has significantly higher ultimate pH, darker color and higher processing characteristics, which might be beneficial for further processed products. Birds with DFD breast meat had experienced severe cold during simulated transport and showed significantly lower core body temperature, blood glucose and liver glycogen compared to birds with normal breast meat. Muscle glycogen reserve at slaughter could not explain the incidence of DFD breast meat, showing that glycogen reserve at slaughter may not be a good predictor for quality attributes of broiler breast meat despite the contrary perception for mammals. Glycolytic potential calculated based on total glucose and lactate concentration at 5 min and 30 h post-mortem could differentiate samples based on quality grouping and correlated well with meat quality attributes. However, it is difficult to explain the buildup in lactate concentration of the DFD breast meat despite the lack of change in the pH of DFD meat post-mortem. The 2 h lairage prior to slaughter of cold-stressed birds caused an increase in the incidence of DFD breast meat, and birds at 5 wk of age were more prone to development of DFD defect under cold environmental conditions. Further assessment of biochemical processes involved in post-mortem

metabolism of DFD meat is required to explain some of the reported results of the current study.

5.6 Connection to the Next Study

There are only a few studies available on thigh muscle of broiler chickens, and no reported study is available to indicate if thigh meat quality attributes are prone to change as a result of cold winter transport similar to breast meat. Therefore, thigh muscle metabolites and meat quality attributes will be investigated in the next study to compare it with breast meat under severe cold temperatures prior to slaughter, with the hypothesis that 'thigh muscle will respond different than breast muscle due to the role they play during transport and also because they are composed of different fiber types'. In addition muscle temperature will be measured after slaughter to investigate if muscle temperature will influence meat quality attributes.

6 THIGH MUSCLE RESPONSE TO COLD-STRESS DURING SIMULATED TRANSPORT

6.1 Abstract

Effect of acute cold exposure during simulated transport was assessed on bird physiology parameters and breast and thigh muscle metabolites and meat quality. One hundred sixty male broilers at two ages (5 and 6 wk) were exposed to temperatures of -9 to -15 (cold-stressed), and +20ºC (control) for 3 h prior to slaughter followed by 0 or 2 h of lairage. Bird physiology parameters (core body temperature (CBT), live shrink and blood glucose) and breast and thigh muscle temperatures were assessed. Muscle metabolites (total glucose and lactate concentrations and glycolytic potential (GP)) were measured at 30 h post-mortem. Meat quality attributes including ultimate pH (pH_u), and color for breast and thigh, water binding capacity (WBC) and processing cook yield (PCY) for breast meat were assessed. Birds were grouped based on experienced temperature to controls and cold-stressed groups (0 to -8, -8 to -11 and -11 to -14ºC). A significant ($P < 0.05$) drop in CBT and breast and thigh muscle temperatures was observed with exposure temperatures below freezing at slaughter. In addition, higher ($P < 0.05$) live shrink and lower blood glucose values were observed as a result of 3 h exposure to temperatures below 0ºC, which was exacerbated as immediate temperature surrounding birds decreased further below -8ºC. A greater effect of environmental temperatures below freezing was observed for thigh meat compared to breast meat, with differences in muscle metabolite concentrations and pH being more pronounced for thigh meat based on temperature groupings compared to breast meat. In addition higher incidence of DFD thigh meat ($pH_u > 6.4$, $L^* < 44$) (85%) was observed compared to DFD breast meat ($pH_u > 6.1$, $L^* < 46$) (42%) as a result of exposure to temperatures below freezing. Results of this study showed that thigh muscle was impacted more severely by cold temperatures during simulated transport compared to the breast meat.

6.2 Introduction

Skeletal muscle is a very heterogeneous tissue composed of a large variety of fiber types being different in metabolic, structural, and contractile characteristics, which might influence the metabolic properties of muscle resulting in variation in muscle metabolism at slaughter and subsequent meat quality (Klont et al., 1998). In poultry, meat is classified to either white or dark, based on the overall color of the meat, which is also related to the proportion of red and white fibers within the muscle (Barbut, 2002). Dark or red muscle, which includes chicken or turkey leg and thigh meat, has a high proportion of red fibers. On the other hand, white muscle such as breast meat is almost entirely composed of white fibers (Barbut, 2002). Red fibers are high in myoglobin, they contract more slowly but have the capacity to operate for a longer period of time; therefore they are equipped with a higher number and larger mitochondria than white fibers (Barbut, 2002). In addition, the higher lipid content allows the fibers to contract for a longer period of time. White fibers, known as glycolytic fibers, have higher amount of glycogen, less myoglobin content, less mitochondria with smaller size and are able to metabolize in either the presence or absence of oxygen, but they contract more rapidly in shorter bursts (Barbut, 2002). These differences in the characteristics of red and white fibers result in differences in post-mortem metabolism between the two fiber types and affect the resultant meat quality.

The majority of studies conducted on the effect of pre-slaughter stress in broilers (Wood and Richards, 1975; Holm and Fletcher, 1997; Berri et al., 2005) and turkeys (Froning et al., 1978; Ngoka et al., 1982) have focused on breast meat quality, perhaps due to higher economic value for breast compared to thigh meat. Only a few studies have looked at the effect of pre-slaughter transport (Debut et al., 2003), crating duration and time (Kannan et al., 1997) and feed withdrawal (Warris et al. 1993) on thigh meat quality. Different effects of pre-slaughter stresses have been reported for breast meat compared to thigh meat, which have not been further explored. Therefore, the aim of this study was to determine the effect of cold-stress during simulated transport on thigh meat quality and compare it with breast meat quality and to assess the incidence of DFD breast and thigh meat as a result of cold exposure prior to slaughter.

6.3 Materials and Methods

A total of 160 male broilers at two ages of 35-37 days old (5 wk old) and 40-42 days old (6 wk old) were exposed to one of the assigned temperatures of -15, -12, -9, and +20ºC in groups of twenty birds at each age for a duration of 3 h as described in Chapter IV. Birds were wing banded, weighed, and orally dosed with temperature logging Thermocron iButtons® (DS1922L iButtonÂ®, Maxim Integrated Products, CA) into the proventriculus to measure core body temperature (CBT) 2 h prior to start of the simulated transport. Birds were taken off feed 7 h prior to start of the trial. I-Buttons were set to measure every minute on high resolution (0.0625). The iButtons were retrieved after birds were euthanized. Birds were placed in a grid system (112 cm x 71 cm) within 2 drawers (10 birds/drawer, first two rows), placed on top of each other in a simulated transport system. The temperature and humidity of each grid location was monitored with Hygrocon iButton® (DS19223 iButtonÂ®, Maxim Integrated Products, CA) data loggers by the minute. Drawers were covered with a mesh lid to keep birds stationary but allowed for air movement until they were placed in the chamber, or while resting after removal from the chamber.

6.3.1 Post chamber treatment

At the end of the exposure duration, 10 birds were randomly chosen from the two drawers and assigned to the 0 h lairage group. Blood was taken from the brachial vein of the 6 wk birds using a heparinised syringe. Five wk birds were not tested for blood glucose due to a shipping complication with the blood glucose kits. The other 10 birds were allowed to sit in a drawer with a mesh top for 2 h before slaughter (referred to as 2 h lairage birds). The same procedure was then followed after the 2 h lairage.

Birds were then transferred to the processing room in groups of 5, where they were processed as described in Chapter IV. Following evisceration, samples were taken from small lobe of the liver to assess liver glycogen content at slaughter as described in Chapter IV. Carcasses were chilled in ice water for 40 min to end point temperature of 7.8±1.8ºC and deboned at approximately 6 h post-mortem with average carcass temperature of 1.3±1.0ºC. Then, breast and thigh samples were placed on Styrofoam trays and stored at 4ºC for 24 h until meat quality attributes were assessed.

6.3.2 Meat quality and muscle metabolites measurement

Breast meat quality was evaluated by measuring color, ultimate pH (30 h post-mortem), water binding capacity (WBC) and processing cook yield (PCY) on the entire set of breast samples as described in Chapter IV. Thigh meat quality was assessed by measuring its pH_u (similar method as breast meat) and color. Color of thigh was measured as lightness (L*), redness (a*), and yellowness (b*) using Minolta Chroma meter (RC-400) with illuminant source C at the 2º setting. The colorimeter was calibrated throughout the study using a standard white ceramic tile. Two readings were performed at 90 º angles to each other from external surface of *Iliotibialis* muscle of thigh meat in an area free of obvious color defects, bruises, and blood spots.

Concentrations of total glucose and lactate were assessed at 30 h post-mortem on all of the breast samples and selected *Iliotibialis* muscle of thigh using similar methods as described in Chapter IV for breast meat. For thigh meat metabolites measurement, 5 samples were selected from each treatment of control and cold-stressed birds at two ages with 0 or 2 h lairage, totaling 40 samples.

6.3.3 Statistical analysis

A completely randomized design with a 4 x 2 x 2 factorial arrangement was employed in this experiment, with 10 birds per treatment combination (n = 160). The model included the main effects of temperature (below -11, -11 to -8, -8 to 0, and control), age (5 and 6 wk old), and lairage (0 or 2 h) and the interactions between them as the main sources of variation in order to analyze the effect of temperature, age and lairage on bird physiology, breast and thigh meat metabolites and quality parameters. Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (SAS Institute, Cary, NC) and results are reported as least square means with their standard deviations. Differences among means were evaluated using the Duncan's multiple comparison test option of SAS. Unless otherwise stated, the means were considered different at a $P \leq 0.05$ by ANOVA.

6.4 Results and Discussion

6.4.1 Effect of cold stress on bird physiology, breast and thigh muscle metabolites and meat quality

In this part of the study 160 male broilers were grouped based on their immediate surrounding temperature to 4 different groups of control, 0 to -8, -8 to -11 and colder than -11ºC. Bird physiology parameters, muscle metabolites and meat quality were evaluated.

6.4.1.1 Bird physiology parameters

The most distinct effect of cold exposure is hypothermia, which occurs when body temperature drops to a degree that birds cannot physiologically regulate their metabolic thermogenesis due to exposure to extreme conditions of cold and wetting (Hunter et al., 1999). Factors such as, body weight, feather coverage, and length of feed withdrawal or amount of energy reserves in the muscles could contribute to the ability of birds to withstand cold temperatures. In the current study average CBT of male broilers dropped significantly ($P < 0.05$) as the immediate temperature surrounding them decreased from 0 to -14ºC compared to the control temperatures (Figure 6.1). Significant ($P < 0.05$) effect of age was observed for CBT, with 5 wk birds showing lower average CBT compared to the 6 wk birds. In addition, the rate and extent of drop in CBT was observed to be greater for the younger birds when immediate temperature surrounding them was below -11ºC (Figure 6.1). The lower lethal body temperature was reported to be ~24ºC for hens and ~21ºC for cocks of approximately 1 year of age (Sturkie, 1946). The majority (50%) of the 5 wk birds exposed to temperatures below -11ºC showed CBT below 24ºC at the end of 3 h exposure time, whereas, within the 6 wk old birds exposed to temperatures below -11ºC none had their CBT dropped below 24ºC at the end of 3 h exposure (Figure 6.2). The higher drop in CBT observed for younger birds compared to the older ones at similar microclimate temperatures was speculated to be the result of bigger size, more feather coverage and therefore more insulation for the 6 wk birds compared to the 5 wk ones. Hence, body mass plays an important role in energy balance in birds (Blem, 2000). The smaller the birds, the more

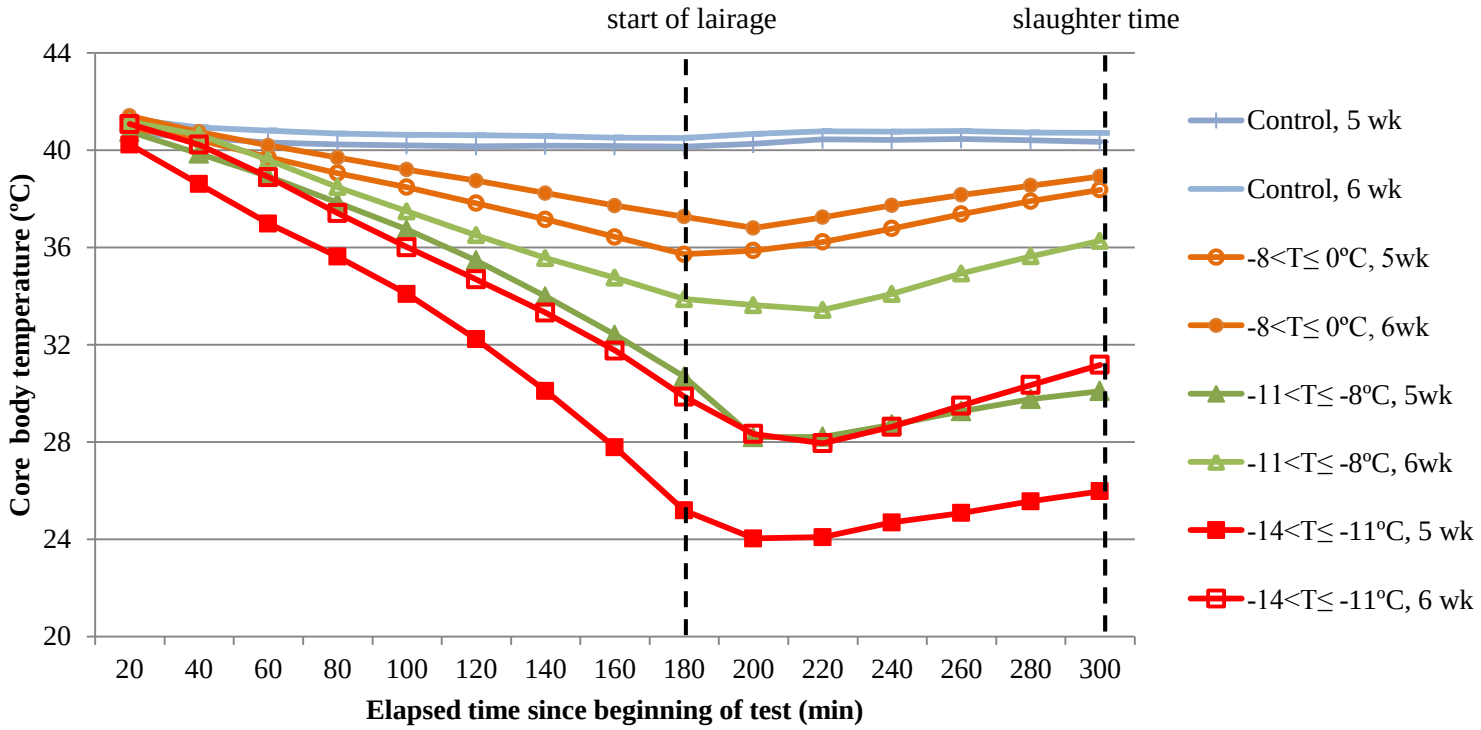
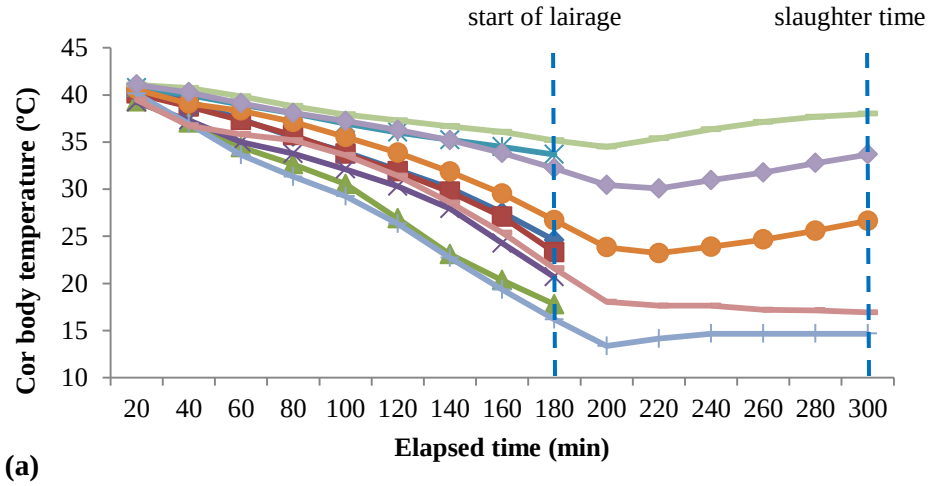
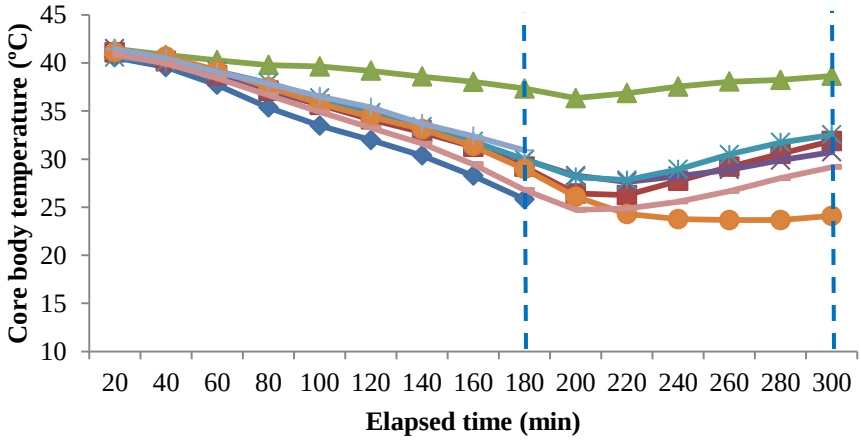


Figure 6-1 Drop in core body temperature over time (3 h chamber and 2 h of lairage).

susceptible they will be to cold temperatures. In addition, less feather coverage and lower fat deposits worsen the situation for younger birds (2.1 kg) compared to the older birds (2.9 kg), which were significantly bigger in size. However, in our previous study (Chapter IV) only 9% of the 5 wk birds exposed to temperatures below -11ºC showed CBT below 24ºC. The 2 h lairage following cold exposure caused a further drop in CBT at the beginning of the rest period, but after 20 min the majority of birds were able to recover their normal CBT partially or completely, depending on their condition when placed into lairage (Figures 6.1 and 6.2).



(a)



(b)

Figure 6-2 Core body temperature (CBT) for the individual birds at 5 (a, 10 birds) and 6 wk (b, 8 birds) during exposure for 3 h to temperatures colder than -11ºC and lairage for 0 or 2 h.

Previous work (Chapter IV) supports the observations of the current study, where a significant decrease in CBT was observed as the exposure temperature dropped from 0 to below -14°C, but it was of a smaller magnitude. Many factors, including biological variability, diet, or other rearing practices, feather coverage, and level of activation or movement during exposure to the cold environment could affect the bird's ability to thermoregulate (Dawson and Whittow, 2000). Experimental settings such as incoming air velocity, feed withdrawal duration, and size of birds were similar between the two studies. Variation in number of the birds per drawer (15 vs. 10 in the previous and current study, respectively) was not considered as the source of variation between the two studies as individual birds were considered experimental units and they were equipped with temperature loggers in their immediate surroundings. Therefore, birds from the previous study may have had greater energy reserves, or better insulation to combat the cold exposure.

Relative humidity (RH) was significantly different between the two studies, with substantially lower RH in the current study (33 and 27% for 5 and 6 wk-old respectively at lower temperature range) compared to the previous study (53 and 71% for 5 and 6 wk respectively at lower temperature range). However, the moisture content of the air was estimated to be very similar based on the psychometric chart (Figure 6.3). At temperature of -10ºC, RH of 20 or 70% results in similar moisture content in the air. It was previously reported by Hunter et al. (1999) that wetting exacerbates cold weather transport, where transport is safe at temperatures as low as -4ºC under dry condition, but it results in a lethal drop of 14.2±5.47ºC in rectal temperature under wet conditions. Therefore, considering the higher RH in the previous study, RH would not contribute to the differences in CBT between the two studies.

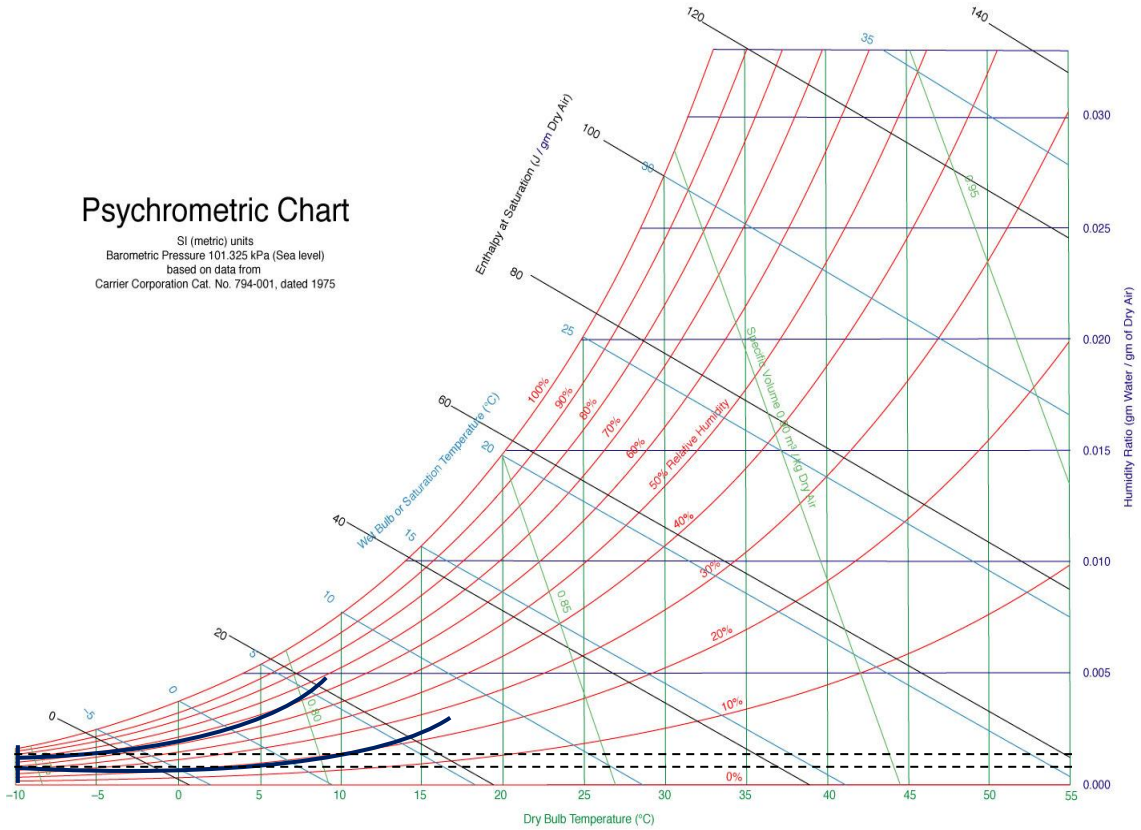


Figure 6-3 Psychometric chart, demonstrating moisture content of the air (vertical axis) based on dry bulb temperature (horizontal axis) and relative humidity (Wikipedia).

Live shrink was observed to be higher for all cold treatment temperatures tested compared to the control temperatures (Table 6.1). Any temperature below 0ºC could result in a high percentage of live shrink and therefore a huge loss to the industry, however among the birds exposed to cold temperatures, live shrink was significantly lower for temperatures below -11 compared to those warmer than -11ºC. Similar results were observed in chapter IV, where all the cold treatment temperatures lead to a significantly higher live shrink (~4%) compared to the control birds (1.4%). However, live shrink was observed to be lower in this study compared to the previous study. A higher live shrink was observed for the 5 wk birds (3.6%) compared to the 6 wk birds (2.3%) in the current study, that could be explained by the fact that these smaller birds

have less energy reserves in their body and are losing more muscle and fat due to the feed deprivation and cold exposure compared to the older birds. The 2 h lairage prior to slaughter caused slightly higher live shrink (by 0.5%) that might be due to both extended feed withdrawal and more energy consumption to recover to normal CBT.

Blood glucose, measured only on the 6 wk birds (2.9 kg), showed a significant incremental decline as the exposure temperature decreased from 0 to -14ºC (Table 6.1). The highest blood glucose values (12.3 mmol/L) were observed for the control birds and the lowest (6.9 mmol/L) were observed for birds exposed to temperatures colder than -11ºC (Table 6.1), which agreed with previously observed results by the same author (Chapter IV).

6.4.1.2 Breast and thigh muscle metabolites and meat quality

It was previously shown that extreme cold temperatures prior to slaughter affects breast muscle energy reserve and subsequent meat quality resulting in darker breast meat with higher pH_u, WBC and PCY (Chapters III and IV). In the present study meat temperature for both breast and thigh measured immediately following slaughter and defeathering showed incremental decline with exposure temperature during 3 h simulated transport, with those exposed to temperatures below -11ºC having the lowest temperature for breast and thigh meats (Table 6.1). But, no difference between breast and thigh meat temperature was observed based on experienced temperature. Birds at 5 wk of age tended to have lower thigh meat temperature compared to the 6 wk birds. The 2 h of lairage resulted in a significant increase in breast meat temperature for all birds and significant increase in thigh meat temperature of the extreme cold-stressed ($-14 \leq T < -11$) birds (data not shown).

Table 6-1 Bird physiology parameters affected by temperature during simulated transport considering different age and lairage.

Variables[1]	Groupings	N	Exp Temp (ºC)	Exp RH (%)	CBT (ºC)	Live shrink (%)	Blood glucose (mmol/L)	Liver glycogen (μmol/g)	Breast temperature (ºC)	Thigh temperature (ºC)
Temperature	20<T≤24	40	21.7±0.7[a]	12.1±0.7[d]	40.5±0.4[a]	1.7±1.0[c]	12.3±0.5[a]	27.8±15.2	40.3±0.8[a]	40.0±0.8[a]
	-8<T≤0	71	-5.37±2.1[b]	23.1±6.5[c]	38.9±1.2[b]	3.4±1.4[a]	10.1±1.2[b]	21.0±15.9	36.3±3.1[b]	37.6±2.1[b]
	-11<T≤-8	31	-9.5±0.9[c]	30.0±6.8[b]	36.9±2.1[c]	3.5±1.5[a]	8.5±1.6[c]	15.2±3.5	33.2±4.6[c]	33.5±4.7[c]
	-14<T≤-11	18	-12.2±1.0[d]	33.5±12.1[a]	34.5±3.0[d]	2.9±1.2[b]	6.9±2.0[d]	18.0±6.5	28.4±6.7[d]	29.3±6.2[d]
***P*-value**			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**NS**	**<0.0001**	**<0.0001**
Age (wk)	5	80	-0.841	25.07	37.76	3.60	---	22.05	35.07	35.68[b]
	6	80	0.520	20.64	39.02	2.30	---	23.13	36.57	37.16[a]
***P*-value**			**0.041**	**<0.0001**	**0.0001**	**<0.0001**	**NA**	**NS**	**NS**	**0.055**
Lairage (h)	0	80	---	---	---	2.71	9.99	21.14	34.88	35.95[b]
	2	80	---	---	---	3.19	10.15	24.08	36.75	36.96[a]
***P*-value**			**NA**	**NA**	**NA**	**NS**	**NS**	**NS**	**<0.0001**	**0.002**
Significant Interactions										
Temperature*age			NS	0.001	NS	NS	NS	NS	NS	NS
Temperature*lairage			NS	NS	NS	NS	0.051	NS	NS	0.015

[a-d] Means with different letters are significantly different at $P < 0.05$.

[1]ExpTemp (experienced temperature measured in birds' immediate surrounding); Exp RH (relative humidity in birds' immediate surrounding); CBT (core body temperature); NS (non-significant); NA (not applicable).

Temperature of simulated transport showed significant ($P < 0.05$) interaction effects with age and lairage on glycolytic potential (GP) and lactate concentration measured at 30 h post-mortem for breast meat (Table 6.2); with 5 wk birds exposed to temperatures below 0ºC having lower breast meat GP_{30h} compared to the control birds (Figure 6.4). But, no difference based on temperature grouping was observed on 6 wk birds breast muscle metabolites (Figure 6.4). Furthermore, the 2 h rest prior to slaughter resulted in lower breast muscle GP, when birds were exposed to temperatures below -11ºC (Figure 6.4). This lower GP with 2 h of lairage prior to slaughter, when birds are exposed to extremely cold temperatures (<-11ºC) can be explained by the fact that, when birds are placed into lairage, they have very little glycogen left to recover their normal CBT, therefore they are burning muscle tissue to recover their homeostasis. In Chapter IV breast meat GP was only affected when temperature during simulated transport dropped below -14ºC and no effect of age at slaughter or lairage duration prior to slaughter was reported on breast muscle GP. In addition, the GP values reported in Chapter IV were higher overall compared to the present data. The lower GP values reported here might be related to lower CBT observed for birds in the current study, indicating that these birds have used more muscle energy reserves to survive the simulated cold transportation and showed more drop in CBT. A better speculation would be that birds in this study had less energy reserves in their muscles prior to treatment.

Exposure temperature during simulated transport showed a significant effect on thigh muscle metabolites, where control birds had the highest thigh muscle lactate and GP at 30 h post-mortem followed by birds exposed to temperatures from 0 to -8ºC, which in turn showed significantly higher GP than birds exposed to temperatures colder than -8ºC (Table 6.3). It should be noted that these differences in muscle metabolites of thigh meat are very pronounced compared to breast meat, with lactate concentration and GP of thigh meat from birds exposed to control temperatures being over twice more than the temperature group of 0 to -8ºC and over five times more than that for birds exposed to temperatures below -8ºC. The two lowest temperature groups were not different from each other in thigh muscle metabolites (Table 6.3).

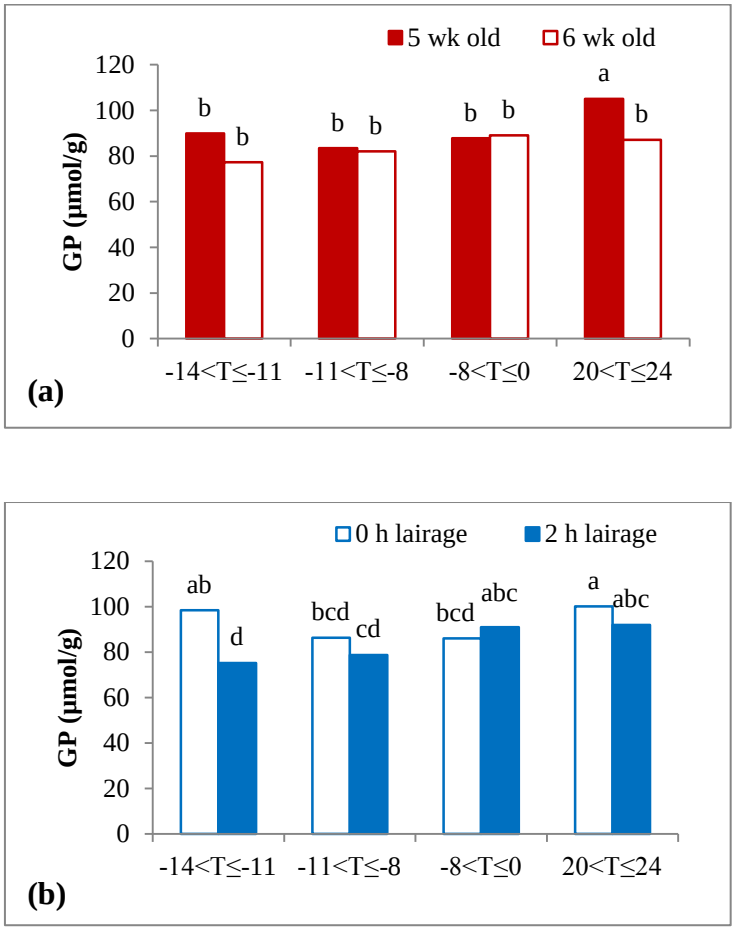


Figure 6-4 Interaction effect of temperature by age (a) and temperature by lairage (b) on glycolytic potential of breast meat from birds exposed to different temperatures during simulated transport.

Breast meat pH_u was significantly ($P < 0.05$) higher for birds exposed to temperatures below -8ºC compared to temperatures between 0 and -8ºC, which in turn was higher than controls (Table 6.2). This agrees with our previous study (Chapter IV). A similar trend was observed for pH_u of thigh meat (Table 6.3) but it was of a much larger magnitude compared to breast meat. For instance, pH_u of breast meat was only different by 0.1 unit between temperature groups, whereas thigh meat of control birds had 0.6 unit lower pH_u compared to thigh meat of birds exposed to temperatures of 0 to -8ºC and 0.8 unit lower than pH_u of thigh meat from birds exposed to temperatures below -8ºC. This difference in pH_u between groups is causative of changes in the subsequent meat quality observed (Tables 6.2 and 6.3).

Table 6-2 Effect of experienced temperature and lairage prior to slaughter and age of birds on breast muscle metabolites and meat quality parameters.

Variables[1]	Groupings	n	pH_u	Lactate 30 h (µmol/g)	GP 30 h (µmol/g)	L*	a*	b*	WBC (%)	PCY (%)
Temperature	20<T≤24	40	6.10±0.11^{c}	88.5±15.2^{a}	96.1±17.5^{a}	47.4±1.8^{a}	3.2±0.7^{b}	4.8±1.2^{a}	33.6±9.2^{c}	94.6±8.7^{c}
	-8<T≤0	71	6.20±0.18^{b}	80.9±16.7^{ab}	88.5±17.7^{ab}	46.0±2.5^{b}	3.8±1.0^{a}	4.1±2.4^{ab}	44.3±11.3^{b}	107.1±15.7^{b}
	-11<T≤-8	31	6.31±0.24^{a}	75.3±15.4^{b}	82.9±16.7^{b}	45.4±3.7^{bc}	4.0±1.4^{a}	3.5±1.9^{b}	50.4±17.7^{a}	120.1±25.4^{a}
	-14<T≤-11	18	6.31±0.22^{a}	76.5±18.4^{b}	84.3±20.6^{b}	44.8±2.5^{c}	4.0±1.0^{a}	3.2±3.2^{b}	46.3±15.1^{ab}	113.9±23.1^{ab}
***P*-value**			**<0.0001**	**0.0003**	**0.0003**	**<0.0001**	**0.0002**	**0.0006**	**<0.0001**	**<0.0001**
Age (wk)	5	80	6.24	82.40	91.38	46.17	3.79	3.63	46.63	113.83
	6	80	6.18	80.09	86.29	46.02	3.63	4.49	39.41	100.62
***P*-value**			**NS**	**NS**	**0.028**	**NS**	**0.084**	**0.031**	**0.003**	**0.0002**
Lairage (h)	0	80	6.19	82.91	90.75	47.10	3.39	4.42	38.67	102.45
	2	80	6.23	79.58	86.91	45.09	4.04	3.70	47.37	112.0
***P*-value**			**0.013**	**0.030**	**0.020**	**<0.0001**	**<0.0001**	**0.009**	**<0.0001**	**<0.0001**
Significant Interactions										
Temperature*age			NS	0.041	0.037	0.011	NS	0.062	NS	NS
Temperature*lairage			NS	0.011	0.016	0.043	NS	NS	0.005	0.071

[a-c] Means with different letters are significantly different at $P < 0.05$

[1] pH_u (ultimate pH measured at 30 h post-mortem); Lactate (30 h) (lactate concentration measured 30 h post-mortem; GP (30 h) (glycolytic potential calculated based on total glucose and lactate concentration at 30 h post-mortem); L* (lightness); a* (redness); b* (yellowness); WBC (water binding capacity); PCY (processing cook yield).

Table 6-3 Effect of experienced temperature and lairage prior to slaughter and age of birds on thigh muscle metabolites and meat quality parameters.

Variables[1]	Groupings	N	Thigh L*	Thigh a*	Thigh b*	N	pH_u	Lactate 30 h (µmol/g)	GP 30 h (µmol/g)
Temperature	20<T≤24	40	50.5±1.8[a]	4.4±0.9[b]	5.2±1.3[a]	20	6.11±0.11[c]	65.6±8.2[a]	73.8±9.9[a]
	-8<T≤0	71	41.6±3.0[b]	6.5±1.6[a]	1.3±1.8[b]	7	6.67±0.29[b]	30.7±17.4[b]	36.3±18.0[b]
	-11<T≤-8	31	40.5±2.0[b]	7.0±1.7[a]	1.0±1.3[b]	10	6.90±0.11[a]	13.4±8.0[c]	17.7±8.3[c]
	-14<T≤-11	18	41.1±2.2[b]	6.4±1.7[a]	1.1±1.3[b]	3	6.95±0.06[a]	14.0±7.3[c]	19.1±9.2[c]
***P*-value**			**<0.0001**	**<0.0001**	**<0.0001**		**<0.0001**	**<0.0001**	**<0.0001**
Age (wk)	5	80	43.37	6.26[a]	1.78[b]	20	6.46	38.35	43.99
	6	80	43.76	5.89[b]	2.65[a]	20	6.47	46.80	54.21
***P*-value**			**NS**	**0.019**	**0.0009**		**NS**	**NS**	**NS**
Lairage (h)	0	80	43.51	5.93	2.22	20	6.51	38.85	44.76
	2	80	43.62	6.21	2.21	20	6.43	46.31	53.43
***P*-value**			**NS**	**NS**	**NS**		**NS**	**NS**	**NS**
Significant Interactions									
Age*lairage			NS	NS	NS		0.040	NS	NS
Temperature*age*lairage			NS	NS	NS		0.033	NS	NS

[a-c] Means with different letters are significantly different at $P < 0.05$

[1]L* (lightness); a* (redness); b* (yellowness); pH_u (ultimate pH measured at 30 h post-mortem); Lactate (30 h) (lactate concentration measured 30 h post-mortem; GP (30 h) (glycolytic potential calculated based on total glucose and lactate concentration at 30 h post-mortem).

Color of both breast and thigh meat were significantly affected by temperature during simulated transport. Birds exposed to temperatures below 0ºC prior to slaughter had significantly ($P < 0.05$) darker (lower L*) and redder breast meat color with higher water binding capacity (WBC) and processing cook yield (PCY) compared to the controls (Table 6.2). No difference in color redness was observed between cold-stressed birds, whereas, birds exposed to temperatures below -11ºC had significantly darker breast meat compared to those exposed to temperatures between 0 and -8ºC, but was not different from the -8 to -11ºC group. Similar effect of cold exposure during simulated transport was reported in the previous study (Chapter IV), however, some of the differences between cold-stressed groups were more pronounced in the previous study. The differences observed between the two studies could be explained by the biological variability between the two flocks of birds and differences observed in muscle energy reserves, which resulted in different pH_u and therefore different ultimate meat quality. However, it is important to note that some of these differences might be due to gender selection, since the previous study used a mixture of male and female broilers versus strictly male broilers being used in the current study.

Age of birds showed a significant effect on some of the meat quality parameters with breast meat of 5 wk birds having significantly higher WBC and PCY compared to the 6 wk birds despite the lack of differences in pH_u between the two ages (Table 6.2). The 2 h lairage prior to slaughter caused a significant ($P < 0.05$) increase in pH_u of breast meat and therefore resulted in breast meat with redder, and less yellow color compared to the breast meat of birds slaughtered immediately after simulated transport (Table 6.2). The interaction of temperature by age was significant for L* value of breast meat (Figure 6.5) with breast meat of control 5 wk birds being significantly lighter than the control 6 wk birds, but no significant difference was observed between ages in breast meat lightness when temperature during simulated transport dropped below freezing. In addition, temperature by lairage showed significant impact on L* and WBC of breast meat (Figure 6.5), with breast meat of birds given 2 h lairage being significantly darker than those slaughtered immediately after treatment when exposed to temperatures below -8ºC. This difference in breast meat lightness of extreme cold stressed birds might be explained by extended feed withdrawal and additional energy

reserve consumption to recover to normal CBT during the 2 h rest prior to slaughter. Breast meat of birds given 2 h of lairage following exposure to temperatures below -8ºC also showed significantly higher WBC (Figure 6.5) and PCY (similar to WBC, data not shown).

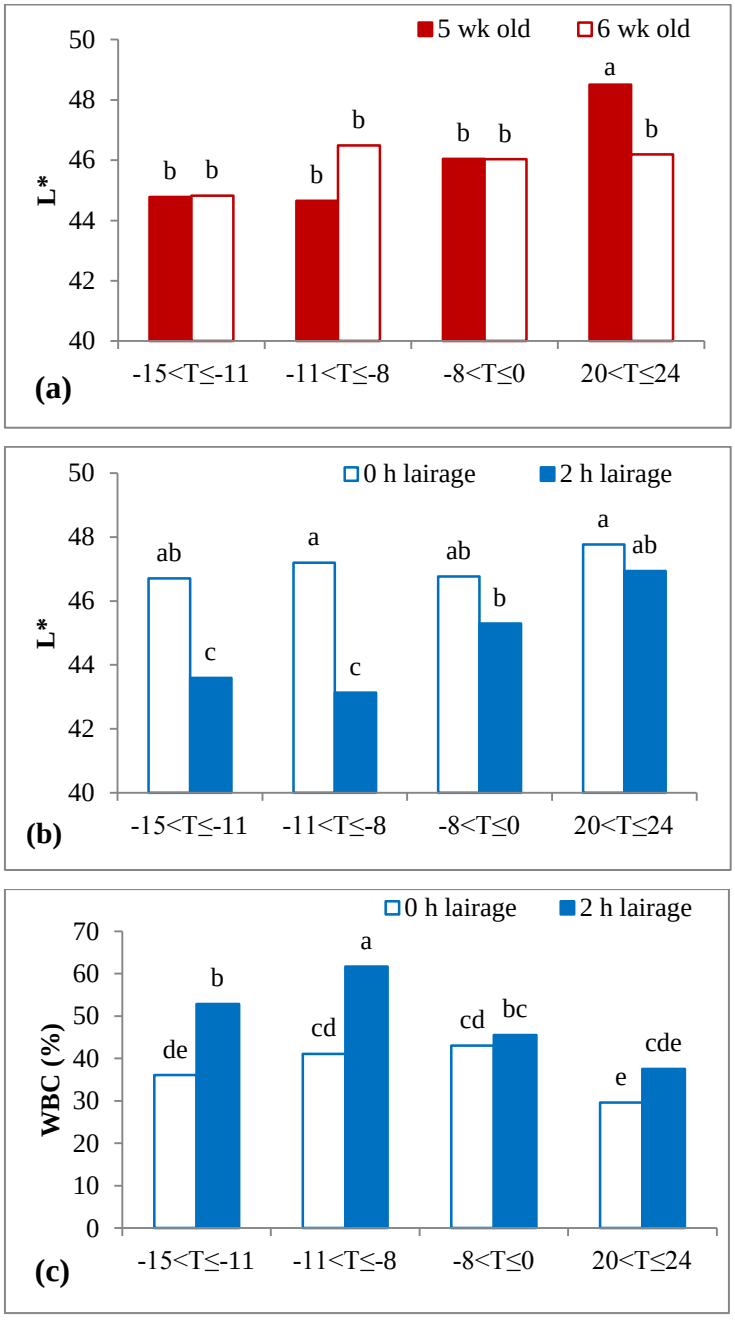


Figure 6-5 Interaction effect of temperature and age on L* value (a) and temperature by lairage on L* value (b) and WBC (c) of breast meat.

Thigh meat of birds exposed to temperatures below freezing was darker, redder and less yellow compared to the control group with no significant differences between the cold temperature groupings (Table 6.3). Bird age had a significant effect on thigh meat redness and yellowness with 5 wk birds having significantly redder and less yellow thigh meat compared to 6 wk birds (Table 6.3). A significant interaction effect of age by lairage was observed for pH_u of thigh meat, with 6 wk broilers showing lower pH_u when given 2 h of lairage compared to 0 h lairage, but no difference in pH_u of 5 wk broiler thigh meat was observed based on lairage period (data not shown). The lower pH_u of thigh meat for the 6 wk rested birds could be related to the replenishment of muscle energy reserves during rest from other energy sources such as fat. Therefore, it can be said that although color is very much pH dependent (r = -89; Table 6.4), it doesn't completely follow the changes observed in pH of thigh meat based on temperature grouping; however, one could argue that the number of samples analyzed for pH_u is much less than those analyzed for color L*. Therefore, in order to draw a conclusion further investigation is required.

Strong positive correlations were observed between experienced temperature during simulated transport with CBT (r = 0.62), blood glucose (r = 0.75), and live shrink (r = 0.46) (Table 6.4). In addition blood glucose was positively correlated to CBT of birds (0.79) (data are not shown). Live shrink showed significant negative correlations with body weight (r = -0.38) and experienced temperature (r = -0.46). Both experienced temperature and CBT showed significant, but low correlations with breast muscle metabolites and meat quality parameters, but high correlations with thigh muscle metabolites and thigh meat color (Table 6.4). Ultimate pH of the breast meat showed significant, negative correlations with simulated transport temperature, CBT, blood glucose, breast meat temperature, lactate and GP (Table 6.4). In addition breast meat pH_u was highly correlated with its color (L*, a*, b*), WBC and PCY (Table 6.4), which agrees with previously reported studies (Berri et al., 2007; Rammouz et al., 2004 b; Zhang and Barbut, 2005; Dadgar et al., 2010; Chapter IV), confirming that pH_u is a good measure of breast meat quality characteristics. Similarly thigh meat pH_u was highly correlated with experienced temperature, CBT, blood glucose, thigh muscle metabolites and thigh meat temperature and color (Table 6.4). The correlations reported

Table 6-4 Pearson correlations for experienced temperature, and physiology parameters with breast and thigh muscle metabolites and meat quality parameters.

	Variables[1]	N	Exp temp (ºC)	CBT (ºC)	Blood glucose (mmol/L)	Liver glycogen (μmol/g)	Breast lactate (30 h, μmol/g)	Breast GP (30 h, μmol/g)	Breast pH_u
Breast	Lac 30(μmol/g)	160	0.30**	0.24**	0.35**	0.01	1.00		
	GP 30 (μmol/g)	160	0.27**	0.21*	0.32**	-0.02	0.97**	1.00	
	pH_u	160	-0.36**	-0.42**	-0.59**	0.03	-0.56**	-0.53**	1.00
	Muscle temp.	160	0.61**	0.85**	0.73**	0.24	0.19*	0.14	-0.33**
	L*	160	0.29**	0.29**	0.19	-0.15	0.42**	0.43**	-0.53**
	a*	160	-0.31**	-0.21*	-0.10	0.22	-0.27**	-0.27**	0.34**
	b*	160	0.25**	0.32**	0.07	-0.10	0.26**	0.25**	-0.36**
	WBC (%)	160	-0.41**	-0.31**	-0.48**	-0.21	-0.46**	-0.42**	0.74**
	PCY (%)	160	-0.41**	-0.38**	-0.56**	-0.12	-0.52**	-0.48**	0.82**
		N	Exp temp (ºC)	CBT (ºC)	Blood glucose (mmol/L)	Liver glycogen (μmol/g)	Thigh lactate (30 h, μmol/g)	Thigh GP (30 h, μmol/g)	Thigh pH_u
Thigh	Lac 30(μmol/g)	40	0.92**	0.78**	0.86**	0.32*	1.00		
	GP 30 (μmol/g)	40	0.91**	0.77**	0.85**	0.31*	0.99**	1.00	
	pH_u	40	-0.93**	-0.77**	-0.87**	-0.26	-0.96**	-0.95**	1.00
	Muscle temp.	149	0.56**	0.86**	0.69**	0.22	0.72**	0.70**	-0.33**
	L*	160	0.85**	0.50**	0.62**	0.15	0.87**	0.86**	-0.89**
	a*	160	-0.53**	-0.30**	-0.27**	0.06	-0.68**	-0.67**	0.69**
	b*	160	0.76**	0.49**	0.51**	0.05	0.80**	0.79**	-0.81**

*, **correlations are significant at $P < 0.05$ and $P < 0.001$ respectively.

[1]Exp temp (experienced temperature); CBT (core body temperature); Muscle temp. (breast or thigh muscle temperature (ºC); Lactate and GP30 (lactate and glycolytic potential at 30 h post-mortem); L*, a*, b* (color lightness, redness and yellowness): WBC, PCY (water binding capacity and processing cook yield in %).

for thigh muscle metabolites and meat quality with physiological parameters and experienced temperature were much higher than those observed for breast meat.

The more pronounced effect of simulated transport temperature on quality parameters of thigh meat compared to breast meat was speculated to be the result of differences in muscle fiber type and role each muscle plays in the body. Thigh and leg muscles are composed partly of oxidative red fibers that are low in glycogen compared to breast muscle being completely composed of glycolytic white fibers that are high in glycogen (Barbut et al., 2005). In addition, breast muscles mostly play structural role compared to leg muscles being involved in maintaining balance in the moving vehicle during transportation (Warriss et al., 1993). Furthermore, it has been shown that slow-twitch and fast-twitch fiber types respond differently to stress in beef *M. longissimus dorsi* muscle, and glycogen depletion happens selectively based on muscle fiber type and its response to stress (Lacourt and Tarrant, 1985). Slow twitch fibers were more prone to glycogen depletion when they were exposed to adrenaline versus mixing stress, but fast-twitch fibers were more prone to glycogen depletion when exposed to mixing stress versus adrenaline stress (Lacourt and Tarrant, 1985). Swatland (1994) also showed that fast contracting fibers were more severely depleted of glycogen due to aggressive behaviour between young bulls. In broilers, thigh meat (slow-twitch) was shown to be more affected by 2 h transport compared to breast meat (fast-twitch) being more affected by physical activity of birds on the shackle line rather than transport stress (Debut et al., 2003). Moreover, a reduction in *Biceps femoris,* but not the *Pectoralis* major glycogen content was reported as a result of increase in transport time (Warriss et al., 1993). Besides, a greater effect of feed withdrawal on *Biceps femoris* but not *P. superficialis* muscles was reported by Warris et al. (1988). Therefore, based on all the aforementioned studies, it is likely that thigh muscle (slow-twitch) was more prone to glycogen depletion compared to breast muscle (fast-twitch) due to differences in fiber type and initial glycogen content and type of stress (cold-stress and transport) prior to slaughter, leading to a larger variation in thigh GP compared to breast meat, which consequently resulted in higher variation in pH_u.

6.5 Conclusion

Exposure to cold temperatures during transportation influenced bird physiology parameters including core body temperature (CBT), blood glucose and live shrinks by causing a significant drop in CBT and blood glucose, and significant increase in live shrink of the birds as a result of exposure to temperatures below freezing. In addition, cold temperatures during simulated transportation had a greater effect on birds at 5 wk of age due to a greater drop in CBT and higher live shrink compared to the 6 wk birds. Furthermore, temperature of thigh and breast muscles dropped incrementally as exposure temperature during simulated transportation decreased from 0 to below -11ºC.

Cold temperatures during simulated transport showed significant effects on breast and thigh muscle metabolites and meat quality parameters, where temperatures below freezing resulted in darker, redder and less yellow breast and thigh meat with higher ultimate pH (pH_u). However the effect of simulated transport temperatures below freezing was greater on thigh meat compared to breast meat, with thigh meat GP being over 5 times lower for extreme cold stressed birds (T<-8ºC) compared to the control birds, whereas this difference in GP based on experienced temperature prior to slaughter was of a much smaller magnitude for breast meat. The pH_u of thigh meat was different by 0.8 unit between controls and extreme cold-stressed birds (T<-8ºC) compared to a difference of 0.2 unit in pH of breast meat. This difference between breast and thigh meat was associated with the difference in fiber type between the two muscles and different response of each muscle to cold stress prior to slaughter.

6.6 Connection to the Next Study

It was observed in Chapter V that energy reserves at slaughter could not explain meat quality parameters and occurrence of DFD defect in broiler breast meat. In addition, glycolytic potential, that is the potential of muscle to produce lactate, could only partially (~50%) explain the variation in broiler breast meat quality. Further, it was shown in the preceding study that breast and thigh muscles respond differently to cold-stress during transport. Therefore a subset of birds tested in the preceding study were selected to further explore if thigh meat is also prone to development of DFD characteristics similar to breast muscle and in order to better understand the basis for development of DFD breast meat in broilers. Therefore the next study was planned to assess DFD development in broiler chickens by monitoring changes in energy reserve post-mortem and assessments of some of the enzymes that might play important roles in post-mortem metabolism.

7 EXPLORING THE BIOCHEMICAL BASIS OF DFD BREAST AND THIGH MEAT IN BROILER CHICKENS

7.1 Abstract

Incidence of cold-induced dark, firm and dry (DFD) breast and thigh meat was investigated in relation to post-mortem metabolism by assessing muscle energy reserves at different times post-mortem. A total of 40 birds with 20 controls (exposed to temperature of +20ºC for 3 h in a simulated transport system) and 20 cold-stressed (exposed to temperatures below 0ºC during 3 h of simulated transport) were selected as part of a larger study. Post-mortem metabolism of breast and thigh muscles was monitored by measuring pH, total glucose and lactate concentrations and calculating glycolytic potential (GP) at different times post-mortem. Activity of AMP-activated protein kinase (AMPK) was assessed on 0 min breast samples. Data was analyzed using ANOVAs option of the GLM procedure of SAS.

Breast and thigh meat were classified based on pH_u and lightness (L*) to normal breast ($5.7 \leq pHu \leq 6.1$; $46 \leq L^* \leq 53$) and thigh ($5.9 \leq pHu \leq 6.4$; $44 \leq L^* \leq 51$) and DFD breast ($pH_u > 6.1$, $L^* < 46$) and thigh ($pH_u > 6.4$, $L^* < 44$). DFD incidence was 85 and 42% for thigh and breast meat from the cold-stressed, compared to 0 and 20% for thigh and breast meat from the control birds. Energy reserves at slaughter were directly related to occurrence of DFD defect in thigh meat compared to breast meat, where DFD thigh meat was completely exhausted of glycogen but not DFD breast meat. Other factors must contribute to DFD development in breast meat, which are not clear at this point. In addition, GP showed much higher correlation with thigh meat quality compared to breast meat quality parameters. GP for breast meat was time sensitive and showed some fluctuations over time, but not for thigh meat. Therefore, the basis of DFD development is different between breast and thigh meat. Total amount of AMPK

was similar for normal and DFD breast samples, but phosphorylated AMPK, indicative of its activity, could not be detected in this study; leading to an unclear role of AMPK in development of DFD breast meat.

7.2 Introduction

Ultimate pH (pH_u), a key factor controlling poultry meat quality, is believed to be mainly related to muscle energy reserves at or around slaughter (Berri et al., 2005, 2007; Hartschuh et al., 2002). Muscle energy reserves can be affected by several ante-mortem factors, including feed withdrawal, stress due to handling, transportation and environmental temperature (Debut et al., 2003). Post-mortem energy demand is provided by ATP through glycogenolysis and glycolysis, which causes a decrease in pH by generating lactate and H^+. The change in metabolites through glycolysis, the involvement of different enzymes controlling glycolytic activity, and the inherent metabolism of different fiber types are critical factors influencing pH decline and meat quality (Lacourt and Tarrant, 1985; Lin et al., 2007; Scheffler and Gerrard, 2007; Shen et al., 2007).

Post-mortem metabolism and factors controlling post-mortem glycolysis have been extensively studied in other species; however, the exact enzymes involved and their specific roles in controlling post-mortem metabolism are still being questioned. Recent studies have highlighted the major role of AMPK in regulating energy levels during post-mortem metabolism (Carling and Hardie, 1989; Jorgensen et al., 2004; Scheffler and Gerrard, 2007). Therefore, evaluation of this enzyme may be useful to increase our understanding of post-mortem metabolism following cold exposure of birds. It is previously reported that AMPK activity controls glycolysis in post-mortem muscle of mice (Du et al., 2005a; Shen and Du, 2005a, b; Shen et al., 2005), pig (Shen et al., 2006) and chicken (Sibut et al., 2008). In the study by Shen and Du (2005a) it was illustrated that AMPK was activated in post-mortem muscle at an early stage, where its activity increased 3 times within the first h after slaughter showing that it promotes post-mortem glycolysis.

According to Shen and Du (2005a) low activity of AMPK was correlated with higher pH_u due to a decrease in glycolytic rate at the glycogenolysis stage, which prevents the drop in pH of the meat. In other studies by Shen and Du (2005b) and Du et al. (2005) a strong correlation between AMPK activation and pH decline in post-mortem mouse *longissimus dorsi* muscle was observed, indicating that AMPK plays an important role in post-mortem regulation of glycolytic muscle. In addition, Shen et al. (2007) showed that if AMPK activation is prevented, glycolysis post-mortem is stopped and an increase in pH_u of the *longissimus dorsi* (LD) muscle of mice was observed. Shen et al. (2006) reported that AMPK reaches its maximum activity more rapidly in pigs that undergo transportation stress compared to the control, or those rested after transport. The severity of PSE in pork has been associated with early post-mortem activation of AMPK and higher activity of this enzyme immediately following slaughter (Shen et al., 2006). In contrast to all the above studies Sibut et al. (2008) showed that breast meat of broilers from a lean line had 3 times higher AMPK activity compared to that of a fat line, and as a result of this higher AMPK activity, a higher pH_u was reported for lean birds. Therefore, studying activity of AMPK in post-mortem metabolism of chicken muscle is necessary to rule out if AMPK activity in chicken is similar or dissimilar to other species and if there is a relationship with AMPK activity and development of DFD breast meat in broilers.

In an earlier study (Chapter V) on the relationship of energy reserves at slaughter and development of DFD breast meat in broiler, it was shown that substrate availability is an important factor, but not the only factor leading to DFD occurrence in chicken breast meat; in addition, the pH of DFD breast meat did not drop despite the lactate produced post-mortem. The objective of this research study was to further investigate the incidence and characteristics of DFD breast meat and compare it to that of thigh meat, which was not previously studied for DFD defect. In addition, role of AMPK was investigated in development of DFD breast meat. It was hypothesized that the lack of activity of AMPK might be the main reason for development of DFD defect in broiler breast meat.

7.3 Materials and Methods

As part of a larger study (Chapter VI), 40 birds were selected from the population of 160 male broilers exposed to control (+20ºC) or cold stressed (-9 to -15ºC) treatment temperatures for duration of 3 h in a simulated transport system as described in Chapter VI. For this study 20 birds were selected from controls and 20 birds were selected from cold stressed birds, equally chosen from 5 (35-37 d old) and 6 (40-42 d old) wk birds that were assigned to 0 or 2 h of lairage prior to slaughter to balance out the effect of these variables.

7.3.1 Bird physiology parameters measurement and sample collection

Bird physiological parameters including core body temperature (CBT), blood glucose (only for 6 wk birds), and live shrink were measured as described in Chapter VI. After welfare assessment birds were transferred to the processing room in groups of 5, where they were processed as described previously. Core samples were taken from the left breast immediately after exsanguination and referred to as 0 min samples. These were immediately frozen in liquid nitrogen and stored at -80ºC for muscle initial pH, glycolytic potential, and AMPK activity analysis. Following 2 min bleeding, the neck was cut using a sharp knife. Right after defeathering core samples were taken from the *Iliotibialis* muscle of the left thigh, frozen in liquid nitrogen and stored at -80ºC for muscle initial pH (~5 min post-mortem) and glycolytic potential analysis. Liver samples (smallest lobe on the left side of the liver) were collected immediately following evisceration (5-10 min post-mortem). Samples were frozen in liquid nitrogen and stored at -80ºC for liver glycogen assessment.

Following evisceration, another set of core samples were taken from breast meat at 20 min post-mortem when birds were placed in a chill tank for duration of 40 min. A third set of core samples were taken from breast and thigh meat upon removal of the carcasses from the chill tank at 60 min post-mortem. Carcasses were deboned at approximately 6 h post-mortem, when another set of core samples were taken from breast meat (6 h post-mortem). Then samples were placed on Styrofoam trays and stored at 4ºC for 24 h, when a third set of core samples were collected from thigh meat and a fifth set of core samples were collected from breast meat.

7.3.2 Meat quality and muscle metabolites measurement

Meat quality was evaluated by measuring color, ultimate pH (30 h post-mortem), water binding capacity (WBC) and processing cook yield (PCY) on breast meat as described in Chapter IV and color and pH_u of thigh meat as described in Chapter VI. Rate of post-mortem metabolism was monitored via measuring pH drop, lactate accumulation, and total glucose concentration at 0, 20 min, 60 min, 6 h and 30 h post-mortem on breast meat and at 5 min, 60 min and 30 h post-mortem on *Iliotibialis* muscle of thigh meat, using similar methods as described in Chapter IV for breast meat metabolites measurements. Initial pH (pH_i) and pH at all times except 30 h post-mortem were measured using an iodoacetate method as described by Sams and Janky (1986).

7.3.3 Classification of samples to quality groupings

Classification of breast and thigh meats were based on ultimate pH (pH_u) and lightness (L*) into normal breast ($pH_u < 6.1$, $L^* > 46$) or thigh ($pH_u < 6.4$, $L^* > 44$) and DFD breast ($pH_u > 6.1$, $L^* < 46$) or thigh ($pH_u > 6.4$, $L^* < 44$) from control (T=20ºC) birds or those exposed to temperatures below freezing prior to slaughter; referred to as cold-stressed birds. These classifications for breast meat were adapted from Barbut et al. (2005); however, thigh meat (*Iliotibialis*) has not been previously classified for quality defects, therefore the classification for this muscle was performed based on pH_u and color within the population in our laboratory.

7.3.4 AMPK activity measurement

AMPK is activated when it is phosphorylated; therefore the activity of this enzyme was evaluated through measurement of the phosphorylated AMPK at α-Thr^{172} (p-AMPK α-Thr^{172}). The level of AMPK activation was evaluated by performing immunoblotting with a phospho-specific antibody on selected breast samples collected at 0 h post-mortem according to Due et al. (2007) and Sibut et al. (2008).

Frozen tissues were ground in liquid nitrogen. Powdered muscle tissue (100 mg) was homogenized in 5x lysis buffer for 10s on ice, using a polytron homogenizer (Kinematica AG 3100, Switzerland). The lysis buffer was prepared according to Du et al. (2007), and contained 150 mM Tris-Cl, 137 mM NaCl, 1mM $CaCl_2$, 1mM $MgCl_2$,

10% glycerol, 2% SDS, 2.5 mM EDTA, 1% triton X-100, phosphatase inhibitors (100 mM sodium fluoride, 10 mM/L sodium phosphate and 1.5 mM sodium orthovanadate). Homogenates were boiled for 5 min with an equal amount of 2x sample loading buffer (37 mM tris-HCl pH 6.8, 4.4% SDS, 2.2% glycerol, and 0.002% Bromophenol Blue), and then centrifuged at 14,000 x g for 10 min. Supernatants were aliquoted and stored at -80°C. Protein concentrations were determined on the homogenates spectrophotometrically at 540 nm by the Biuret method using the Biuret reagent (1:10) following a 30 min incubation at room temperature. Muscle lysates (80 µg of protein) were subjected to 10% (wt/vol) SDS-PAGE under reducing conditions and electrotransferred at 100v for 1h at 4°C. Membranes were blocked in 1X TBS (Tris Buffered Saline) with 5% w/v non-fat dry milk that binds/blocks non specific bands, improving conditions for antibodies that have a high affinity for protein. The membranes were then incubated overnight at 4°C with appropriate primary antibodies at a final dilution of 1:1000 in Tris-buffered saline (TBS, 2 mM Tris-HCl, pH 8, 15 mM NaCl, pH 7.6) containing 0.1% Tween-20 and 5% non-fat dry milk powder. Rabbit polyclonal antibodies to AMPK_α and to p-AMPK α-Thr^{172} were obtained from Cell Signalling (Cell Signalling Technology Inc., Pickering, ON). Mouse monoclonal anti-human vinculin was purchased from Sigma-Aldrich, Co (CHEMIE GmbH, St. Louis. MO, USA). Membranes were washed 5 times and were then incubated with IRDye 800CW Goat anti-Mouse, and IRDye 680CW Goat anti-Rabbit Secondary Antibodies (LI-COR Biosciences, Mandel Scientific Company Inc., Guelph, ON). Bands were visualized by infrared fluorescence using an Odyssey Imaging System (LI-COR Biosciences, Mandel Scientific Company Inc., Guelph, ON) and quantified using Odyssey infrared imaging system software (Application software, version 1.2).

7.3.5 Statistical analysis

A completely randomized design with 4 x 2 x 2 factorial arrangement was employed with unequal number of samples per group considering the main effects of quality (DFD and normal from control or cold-stressed birds), age (5 and 6 wk old), and lairage (0 or 2 h) and the interactions between them as the main sources of variation in

order to assess effect of quality groupings on pH, muscle metabolites and meat quality of breast and thigh meats. Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of SAS (SAS Institute, Cary, NC) and results are reported as least square means with their standard deviations. Differences among means were evaluated using the Duncan's multiple comparison test option of SAS. Unless otherwise stated, the means were considered different at a $P \leq 0.05$ by ANOVA.

7.4 Results and Discussion

7.4.1 Characteristics and incidence of cold-induced DFD breast and thigh meat

Poultry meat is recognized by having both white (breast) and red (thigh and leg) muscles. These differ in pH_u, where red leg muscle is reported to reach pH_u of 5.9-6.0 within 2-3 h post-mortem, but white breast muscle pH is reported to decline beyond 24 h post-mortem to reach values of 5.4-5.6 (Lyon and Buhr, 1999). However, the normal pH of breast meat (5.8 to 6.1) was observed to be higher than literature norms throughout this thesis, and several other studies have also reported higher pH_u for breast (5.7 to 6.0) (Barbut et al., 2002, 2005; Debut et al., 2003) and thigh (6.0 to 6.3) (Debut et al., 2003) muscles.

Birds exposed to the control temperature did not have any thigh meat with DFD characteristics, but approximately 20% DFD breast meat was observed within the control birds. On the other hand, 85% of the thigh meat from cold-stressed birds showed DFD characteristics compared to 42% of the breast meat. These reported incidences are also representative of the population (160 male broilers with 40 controls and 120 cold-stressed birds) as shown in Figure 7.1. It is interesting to note that none of the control birds showed thigh meat with DFD properties, whereas the majority of birds under cold exposure showed thigh meat with DFD properties. However, in Chapter V, no incidence of DFD breast meat was observed within the control birds. Nevertheless, no significant differences ($P < 0.05$) were observed in experienced temperature, core body temperature (CBT), and live shrink (%) between control birds with normal or DFD breast meat or

between cold-stressed birds with normal or DFD breast meat (Table 7.1). However, in Chapters III and V, cold-stressed birds that had breast meat with DFD properties showed significantly lower CBT compared to those having normal breast meat, but in this study although cold-stressed birds with DFD breast meat showed lower CBT, it was not different from cold-normal meat birds, but was lower than control birds. Liver glycogen content was significantly higher in control birds developing DFD breast meat compared to all other groups (Table 7.1). Therefore, one might speculate that these control birds did not use their liver glycogen reserve for some reason and relied mainly on muscle glycogen reserves, leading to development of DFD breast meat.

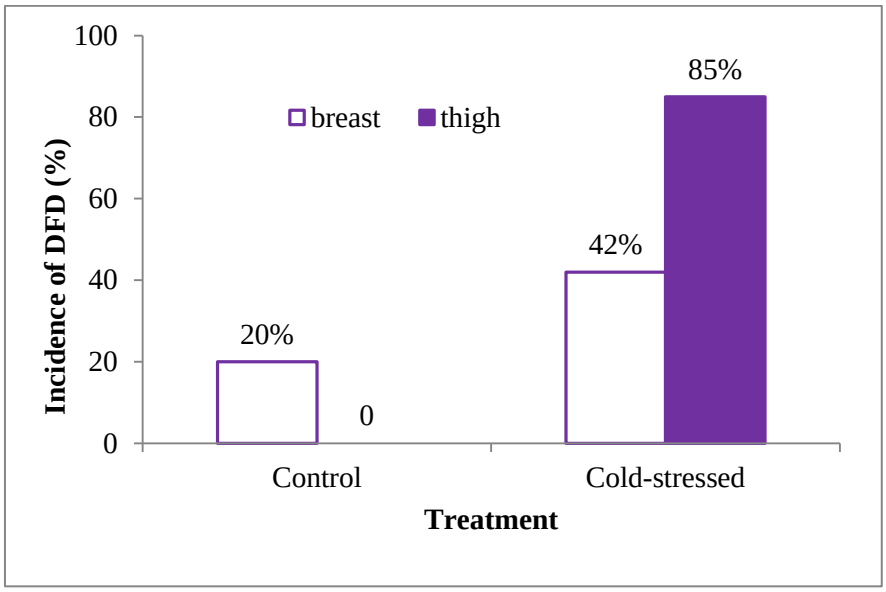


Figure 7-1 Incidence of DFD breast and thigh meat within control and cold-stressed birds (n = 160).

Table 7-1 Bird physiology and breast meat quality parameters from birds exposed to control and cold treatments during simulated transport and classified based on breast meat quality considering age effect and lairage prior to slaughter.

Variables[1]		n	Exp temp (ºC)	CBT (ºC)	Live shrink (%)	Liver glycogen (μmol/g)	Breast L*	Breast a*	Breast b*	WBC (%)	PCY (%)
Quality grouping	Control-normal	15	21.4±0.9^{a}	40.4±0.4^{a}	1.8±1.0^{b}	24.1±10.1^{b}	47.8±1.5^{a}	3.1±0.7^{b}	4.9±1.3^{a}	31.4±8.4^{b}	92.5±7.1^{b}
	Control-DFD	5	21.9±0.8^{a}	40.5±0.5^{a}	1.4±1.0^{b}	38.8±23.3^{a}	45.0±0.8^{b}	4.0±0.4^{b}	4.1±1.1^{a}	34.8±10.2^{b}	98.8±13.4^{b}
	Cold-normal	13	-7.8±2.4^{b}	37.4±3.0^{b}	3.4±1.1^{a}	16.8±4.4^{b}	46.9±2.4^{ab}	3.6±0.9^{b}	4.0±1.9^{a}	40.2±13.0^{b}	106.2±19.5^{b}
	Cold-DFD	7	-9.6±2.2^{b}	36.5±0.9^{b}	3.6±2.1^{a}	21.6±19.4^{b}	41.7±3.1^{c}	5.4±1.6^{a}	1.3±1.4^{b}	71.8±17.7^{a}	150.1±30.1^{a}
***P*-value**			**<0.0001**	**0.0005**	**<0.0001**	**0.007**	**<0.0001**	**0.008**	**0.001**	**<0.0001**	**<0.0001**
Age	5 wk	20	5.98	38.19	3.38	22.63	46.37	3.82	3.64	45.56a	114.56
	6 wk	20	7.12	39.26	1.77	23.66	45.83	3.76	4.14	37.92b	101.07
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.019**
Lairage	0 h	20	6.27	38.58	2.52	21.80	47.35a	3.40b	4.55a	35.73b	101.26b
	2 h	20	6.83	38.90	2.63	24.49	44.85b	4.18a	3.23b	47.74a	114.37a
***P*-value**			**NS**	**NS**	**NS**	**NS**	**0.015**	**NS**	**NS**	**0.032**	**NS**
Significant Interactions											
Quality grouping*age			NS	NS	NS	NS	NS	NS	0.043	NS	NS
Quality grouping *lairage			NS	NS	NS	0.011	NS	NS	NS	NS	NS

[a-d] Means±SD with different letters are significantly different within a column.

[1]Exp Temp (temperature in birds' immediate surroundings during 3 h exposure); CBT (average core body temperature of birds during 3 h simulated transport); L*, a*, b* (color lightness, redness and yellowness); WBC (water binding capacity); PCY (processing cook yield).

From a meat quality stand point, no difference in redness or yellowness, WBC and PCY was observed between DFD breast meat of the control birds and normal breast meat of either control or cold-stressed birds. Yet, DFD breast meat of the control birds was significantly darker from normal breast meat of the control birds, but not normal breast meat of the cold-stressed birds. Nevertheless, DFD breast meat from the cold-stressed birds was the darkest, reddest, and yellowest of all with the highest WBC and PCY compared to all other groups (Table 7.1). These characteristics of DFD breast meat was previously reported in Chapter V of this thesis and elsewhere (Barbut et al., 2005; Zhang and Barbut, 2005).

Age of birds showed significant ($P < 0.05$) effect on PCY, with 5 wk birds showing higher PCY compared to the 6 wk birds (Table 7.1). Interaction of age and quality grouping was significant for color b*, with normal breast meat from 6 wk birds having significantly yellower color compared to normal breast meat from 5 wk birds, when exposed to cold temperatures (5.0 compared to 2.8 respectively), but no significant effect of age was observed within other quality groups. In addition, lairage showed significant ($P < 0.05$) effect on color L* and WBC of breast meat. Breast meat of birds assigned to 2 h lairage was significantly darker (44.8 vs. 47.5) in color, and had higher WBC (48.0 vs. 36.0) compared to breast meat of birds slaughtered immediately after the trial (Table 7.1). Interaction effect of DFD grouping and lairage was observed on liver glycogen, in that cold-stressed birds with DFD breast meat had higher liver glycogen when given 2 h lairage (25 μmol/g) compared to birds slaughtered immediately following cold exposure (14 μmol/g), but no difference was observed in liver glycogen content based on lairage duration when subsequent breast meat was normal regardless of temperature treatment. Furthermore, interaction effects of lairage and quality groupings were significant on pH measured at 20 min post-mortem and total glucose at slaughter; however, no conclusive results could be drawn from these interactions (data not shown).

Thigh meat showed more distinct differences based on quality grouping compared to breast meat. Cold-stressed birds that developed DFD thigh meat had experienced significantly lower temperature in their immediate surroundings during 3 h

of simulated transport and had lower CBT compared to those developed normal thigh meat (Table 7.2). No difference in liver glycogen content was observed based on thigh meat quality grouping. DFD thigh meat was significantly darker, and yellower compared to normal thigh meat from both control and cold-stressed birds. The normal thigh meat from the cold-stressed birds was significantly darker compared to that of control birds, but was not different in redness or yellowness from the controls (Table 7.2).

Significant effect of age was observed on thigh meat pH measured at 1 h (Table 7.2) and thigh muscle metabolites measured at 30 h post-mortem, with 5 wk birds showing lower pH (6.61) compared to 6 wk birds (6.73) at 1 h post-mortem. In addition thigh meat of 5 wk birds showed significantly lower total glucose (2.82 vs. 3.70), lower lactate (38.4 vs. 46.8) and lower GP (44.0 vs. 54.2) at 30 h post-mortem compared to the thigh meat of 6 wk birds. Furthermore, the 2 h lairage caused significant decrease in thigh meat initial pH (6.63 vs. 6.76) (Table 7.2) and significant increase in ultimate lactate concentration (46.3 vs. 38.8) and GP (53.4 vs. 44.8). Significant interaction of thigh meat quality grouping with lairage was observed on total glucose measured at 30 h post-mortem and color a*. Cold-stressed birds that developed DFD thigh meat showed higher a* value when given 2 h of lairage (8.3 vs. 6.6) compared to 0 h lairage. In addition, when cold-stressed birds that had normal thigh meat were assigned to 2 h lairage, it resulted in higher glucose at 30 h post-mortem compared to normal thigh meat of birds slaughtered immediately following cold exposure.

Table 7-2 Thigh meat quality parameters for DFD or normal meat from birds exposed to control or cold temperatures prior to slaughter.

Variables[1]		n	Exp Temp (ºC)	CBT (ºC)	Liver glycogen (µmol/g)	L*	a*	b*	pH $_{5min}$	pH$_{1h}$	pH $_{30h}$
Quality grouping	Control-normal	20	21.6±0.9	40.5±0.4^{a}	27.8±15.2	50.1±2.0^{a}	4.8±0.8^{b}	5.2±1.6^{a}	6.53±0.12^{b}	6.49±0.13^{b}	6.11±0.11^{c}
	Cold-normal	3	-4.5±0.5	39.8±0.9^{a}	19.0±3.4	45.9±2.6^{b}	5.9±1.5^{ab}	3.3±1.8^{a}	6.62±0.09^{b}	6.58±0.08^{b}	6.44±0.23^{b}
	Cold-DFD	17	-9.2±1.9	36.6±2.3^{b}	18.4±12.7	39.8±3.1^{c}	7.4±2.0^{a}	0.2±2.0^{b}	6.89±0.13^{a}	6.91±0.13^{a}	6.90±0.12^{a}
***P*-value**			**<0.0001**	**<0.0001**	**0.207**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Age (wk)	5	20	5.98	38.19	22.63	45.30	6.09	2.47	6.68	6.61b	6.46
	6	20	7.12	39.26	23.66	45.54	5.85	3.47	6.71	6.73a	6.48
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.0001**	**NS**
Lairage (h)	0	20	6.27	38.58	21.80	45.80	5.74	3.26	6.76a	6.70	6.51
	2	20	6.83	38.90	24.49	45.05	6.20	2.67	6.63b	6.64	6.43
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.0002**	**NS**	**NS**
Significant Interactions											
Quality grouping *age			NS	NS	NS	NS	NS	NS	0.011	NS	NS
Quality grouping*lairage			NS	NS	NS	NS	0.037	NS	NS	NS	NS

[a-d] Means±SD with different letters are significantly different within a column.

[1]Exp Temp (temperature in birds immediate surroundings during 3 h exposure); CBT (core body temperature of birds averaged during 3 h simulated transport); L*, a*, b* (color lightness, redness and yellowness); pH (pH measured at different times post-mortem on *Iliotibialis* muscle).

Thigh meat color measurements were more influenced by simulated transport temperature and CBT compared to the breast meat (Table 7.3). In addition pH and GP of muscle measured at different times post-mortem correlated better with thigh meat color parameters compared to breast meat color parameters (Table 7.3). The higher correlations observed for thigh meat quality attributes with muscle metabolites suggest that quality parameters of thigh muscle may be more directly influenced by change in muscle metabolite concentrations. Therefore, quality characteristics of thigh could be better predicted based on stress prior to slaughter (transportation and cold temperature) and also based on muscle metabolites compared to breast meat.

Table 7-3 Pearson correlations of pH and GP measured at different times post-mortem with color attributes of breast and thigh meat (n=40).

		ExpTemp[1] (ºC)	CBT (ºC)	pH_i	pH_{1h}	pH_{30h}	GP_i	GP_{1h}	GP_{30h}
Breast	L*	0.31*	0.29	-0.42*	-0.31*	-0.52**	0.39	0.61***	0.54**
	a*	-0.36*	-0.25	0.08	0.17	0.51**	-0.33	-0.56**	-0.47*
	b*	0.41*	0.54**	-0.18	-0.18	-0.48*	0.33	0.50**	0.40*
Thigh	L*	0.86***	0.63***	-0.73***	-0.80***	-0.89***	0.92***	0.88***	0.87***
	a*	-0.64***	-0.54**	0.53***	0.58***	0.67***	-0.68***	-0.68***	-0.66***
	b*	0.79***	0.66***	-0.64***	-0.65***	-0.80***	0.79***	0.74***	0.79***

*,**,*** Correlations are significant at $P < 0.05$, $P < 0.001$, and $P < 0.0001$ respectively.

[1]Exp Temp (temperature in immediate surroundings in ºC), CBT (core body temperature in ºC); pH_i and GP_i (initial pH and glycolytic potential measured at 0 and 5 min post-mortem on breast and thigh muscles respectively); $pH_{1h,\ 30h}$ and $GP_{1h,\ 30h}$ (pH and GP measured at 1 h and 30 h post-mortem)

In addition, much higher correlations were observed within GP measured at different times post-mortem for thigh meat compared to breast meat (data not shown). All these observations are indicative of differences between muscles perhaps due to

different fiber types and different responses to cold exposure. Therefore GP could predict post-mortem meat quality of thigh oxidative red muscle (greater proportion of red fiber) to a greater extent than breast glycolytic muscle (composed mainly of white fibers).

The greater effect of cold stress prior to slaughter on thigh muscle compared to the breast muscle and higher incidence of DFD thigh meat compared to DFD breast meat for the cold-stressed birds are very interesting observations. Based on the current literature and as discussed earlier (Chapter VI) these differences between the two muscles could be mainly related to the difference in fiber type and its response to type of stress and role each muscle play during stress. Thigh *Iliotibialis* muscle (higher red fiber) has lower glycogen content compared to breast *Pectoralis* major (homogeneously white fiber) muscle, and it is shown that glycogen depletion happens selectively based on muscle fiber type and its response to the type of stress (Lacourt and Tarrant, 1985). In addition greater effect of transport stress (Debut et al., 2003; Warriss et al., 1993), crating duration (Kannan et al., 1997), and feed withdrawal (Warriss et al., 1993) was reported for thigh compared to breast meat which is more affected by physical activity prior to slaughter.

These results further confirm that the Klont et al. (1998) observation of selective response of different fiber types to stress and glycogen depletion is also true for chicken species. In addition it was reported that muscles tend to have meat with more DFD characteristics with more than 30% glycogen depletion in their fast-twitch (type IIB) fibers (Karlsson et al., 1994). The higher variation in pH of the thigh meat might be explained by the fact that pH is more variable in muscles with a higher oxidative capacity (Klont et al., 1998) due to the non-linear relationship between glycogen content and pH_u (Fernandez and Tornberg, 1991). Therefore, in the next section of this study the post-mortem energy metabolism in breast and thigh meat with normal and DFD quality characteristics was investigated in order to explain some of the differences observed between the two muscles.

7.4.2 Post-mortem metabolism in relation to development of DFD defect in breast and thigh muscles

In order to understand the mechanism involved in the development of DFD breast and thigh meat, it is important to know post-mortem metabolism pathways and enzymes involved in glycogenolysis and glycolysis which was discussed in detail in Chapter II of this thesis. In the current study, rate of post-mortem metabolism was monitored by assessing pH drop and changes in glucose and lactate concentrations from time 0 to 30 h post-mortem in order to explore the basis for development of DFD defect in breast and thigh muscles of broiler chickens.

No significant difference was observed in breast meat pH until 6 h post-mortem regardless of quality groupings (Figure 7.2). At 6 and 30 h post-mortem, only the pH of DFD breast meat from the cold-stressed birds was significantly higher than the other groups. On the other hand, DFD thigh meat showed significantly higher pH from 5 min post-mortem onward compared to normal thigh meat of both control and cold-stressed birds which were not significantly different from each other until 30 h post-mortem (Figure 7.2).

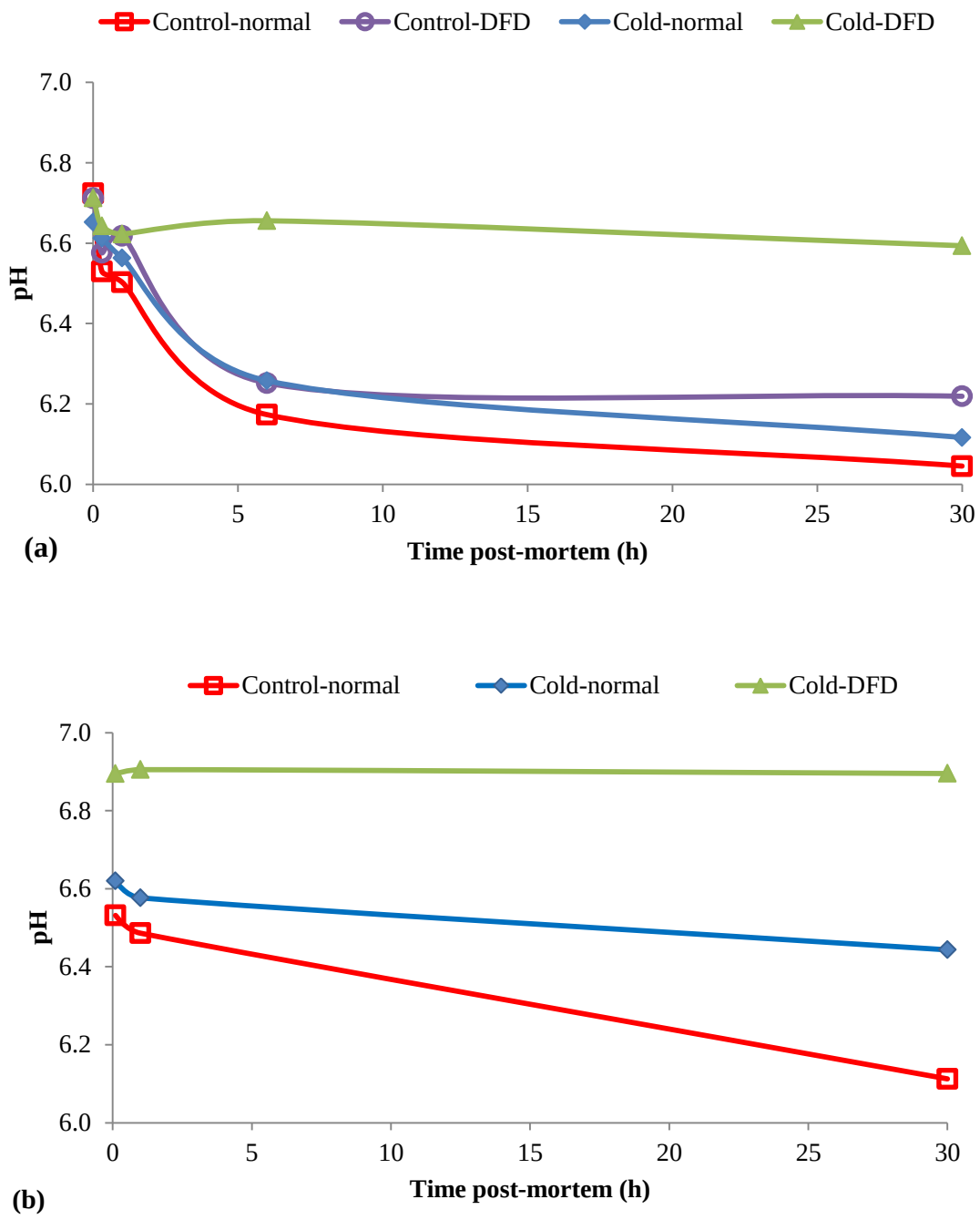


Figure 7-2 Post-mortem pH drop for breast (a) and thigh (b) muscles categorized based on normal or DFD meat from birds exposed to control or cold temperatures prior to slaughter.

Table 7-4 Breast meat pH measured at different times post-mortem for normal and DFD breast meat from control or cold exposed birds considering age effect and lairage prior to slaughter.

Variables		n	pH $_{0min}$	pH $_{20min}$	pH $_{1h}$	pH $_{6h}$	pH $_{30h}$
Quality groupings	Control-normal	15	6.72±0.08	6.53±0.09^{b}	6.56±0.14	6.17±0.11^{b}	6.05±0.11^{b}
	Control-DFD	5	6.71±0.06	6.58±0.09^{ab}	6.61±0.06	6.25±0.10^{b}	6.22±0.09^{b}
	Cold-normal	13	6.65±0.08	6.61±0.11^{ab}	6.56±0.08	6.26±0.25^{b}	6.12±0.30^{b}
	Cold-DFD	7	6.71±0.12	6.64±0.10^{a}	6.6±0.16	6.66±0.23^{a}	6.59±0.19^{a}
***P*-value**			**NS**	**0.071**	**0.540**	**0.001**	**0.008**
Age (wk)	5	20	6.68	6.61a	6.52b	6.36a	6.20
	6	20	6.71	6.56a	6.60a	6.23b	6.18
***P*-value**			**NS**	**0.049**	**NS**	**NS**	**NS**
Lairage (h)	0	20	6.67b	6.57	6.53	6.25	6.16
	2	20	6.72a	6.59	6.59	6.35	6.22
***P*-value**			**0.01**	**NS**	**NS**	**NS**	**NS**
Significant Interactions							
Quality groupings*lairage			NS	0.005	NS	NS	NS

$^{a-d}$ Means±SD with different letters are significantly different within a column.

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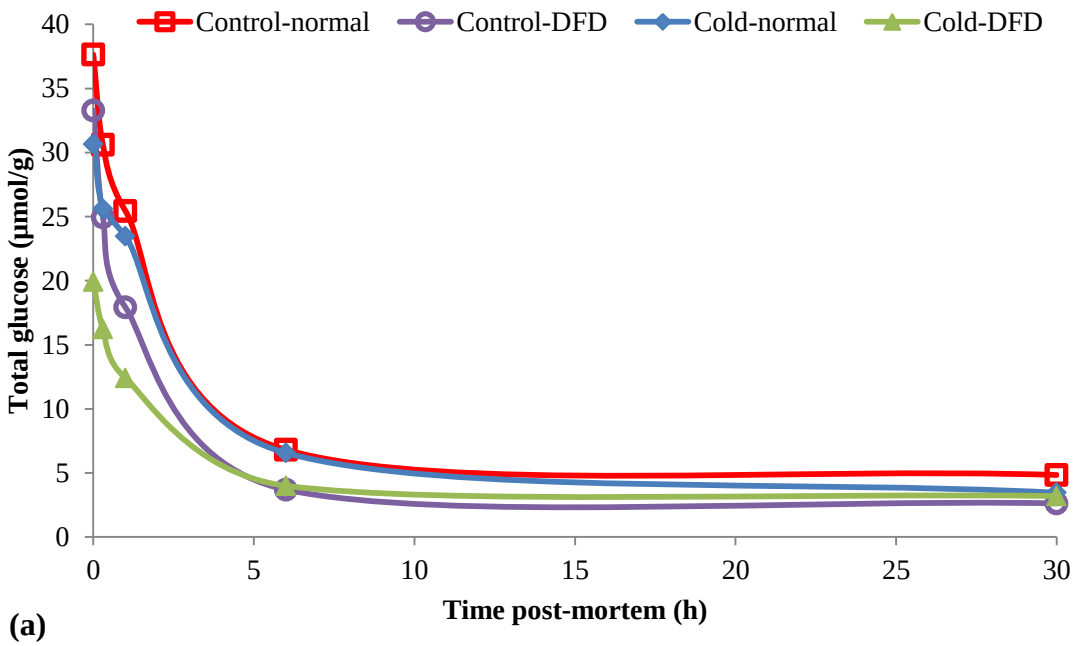
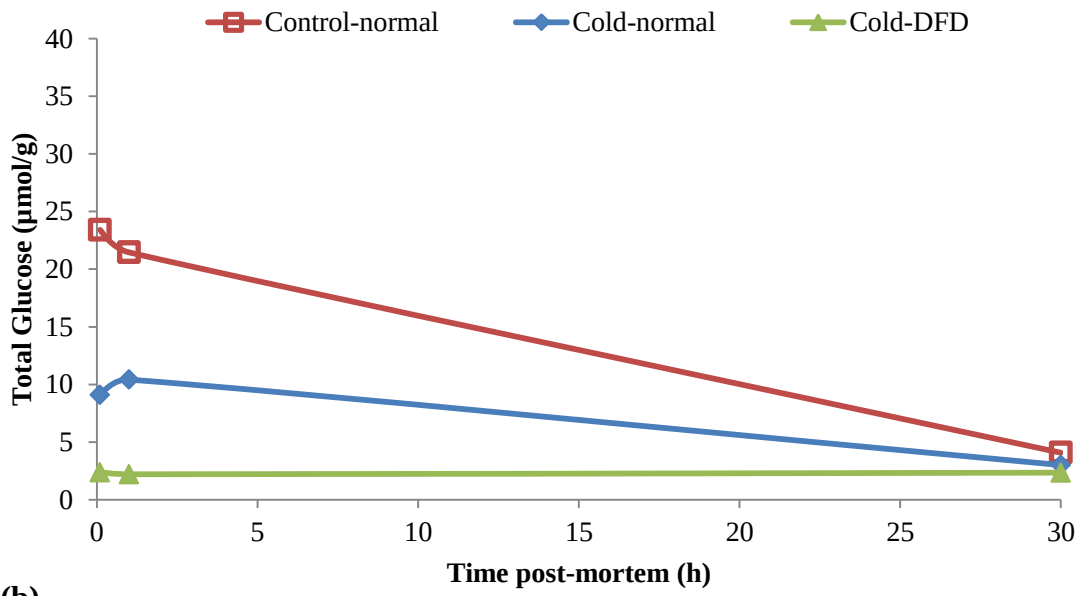
Total glucose was almost exhausted at 5 min post-mortem in DFD thigh meat (2 μmol/g) compared with normal thigh meat from both control and cold-stressed birds (23 and 9 μmol/g respectively), suggesting that the lack of substrate availability resulted in DFD in thigh meat (Figure 7.3). The normal thigh meat from the cold-stressed birds showed significantly lower glucose at 5 min and 1 h post-mortem compared to the normal thigh meat from the control birds, but apparently this energy reserve was enough to produce normal quality thigh meat. However, breast meat total glucose at slaughter was lower only in DFD meat (20 μmol/g) from the cold-stressed birds, and not DFD breast meat from the control birds (33 μmol/g) compared to normal breast meat from either control or cold-stressed birds (38 and 31 μmol/g respectively) (Table 7.5). By 1 h post-mortem DFD breast meat from both control and cold-stressed birds showed significantly lower total glucose compared to their normal counterparts. However, by 6 h post-mortem total glucose was almost completely exhausted in all of the breast samples regardless of quality defect (Table 7.5; Figure 7.3). It is interesting to note that, despite any significant difference in total glucose concentration at slaughter, or any time post-mortem some samples produce normal and some produce DFD characteristics. Therefore role of glycogen reserve in breast meat remain unresolved.

Overall, a more dramatic effect of cold stress prior to slaughter was observed on total glucose reserves for thigh compared to breast muscle (Table 7.6; Figure 7.3). One speculation here is that thigh has less glycogen reserve compared with breast muscle to start with, and slow-twitch fibers are more prone to transport stress (Debut et al., 2003), or extended transport time and feed withdrawal (Warriss et al., 1993) compared to breast muscle. In addition, leg muscles are involved in maintaining balance in the moving vehicle during transportation or even during simulated transport due to vibration, and last but not least it is reported that the fibers responsible for shivering are mostly the slow-contracting type; therefore animals exposed to severe cold condition prior to slaughter may have less glycogen in these fibers [Lupandin and Poleshchuk (1979) cited in Swatland (1994)].



(a)



(b)

Figure 7-3 Total glucose (TG) measured at different times (0 to 30 h) post-mortem in *Pectoralis* major (a) and *Iliotibialis* (b) muscles.

Table 7-5 Breast meat total glucose (TG) and lactate (Lac) concentrations measured at different times post-mortem for normal and DFD breast meat from control or cold exposed birds considering age effect and lairage prior to slaughter.

Variables[1]			TG $_{0min}$	TG $_{20min}$	TG $_{1h}$	TG $_{6h}$	TG $_{30h}$	Lac $_{0min}$	Lac $_{20min}$	Lac$_{1h}$	Lac $_{6h}$	Lac $_{30h}$
Quality groupings	Control-normal	15	37.6±8.7^{a}	30.6±7.9	25.4±3.6^{a}	6.8±2.4	4.8±3.2	27.1±11.4	37.9±10.5	35.6±8.7	76.5±8.5^{a}	93.5±16.5^{a}
	Control-DFD	5	33.3±6.4^{a}	24.9±4.7	17.9±2.8^{bc}	3.7±0.8	2.6±0.4	24.9±5.2	40.5±8.6	40.9±10.5	71.6±16.1^{a}	73.0±5.2^{b}
	Cold-normal	13	30.7±9.9^{a}	25.6±6.9	23.5±7.1^{ab}	6.6±3.5	3.5±1.2	25.4±11.3	34.9±11.9	34.5±7.8	79.8±16.9^{a}	78.5±19.5^{ab}
	Cold-DFD	7	19.9±8.2^{b}	16.2±10.7	12.4±7.6^{c}	4.0±1.1	3.2±1.0	27.2±7.2	32.3±2.8	33.2±13.3	48.3±16.4^{b}	53.6±17.0^{c}
***P*-value**			**0.021**	**0.380**	**0.022**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.0072**	**0.0057**
Age (wk)	5	20	33.76	26.06	20.60	5.54	4.71a	25.66	32.97b	36.36	69.83	82.01
	6	20	29.70	25.46	22.57	6.16	2.94b	26.93	39.56a	34.60	74.24	76.15
***P*-value**			**NS**	**0.049**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Lairage (h)	0	20	31.92	25.64	23.02	6.18	3.56	25.25	36.55	35.70	71.87	82.37
	2	20	31.53	25.88	20.14	5.52	4.10	27.34	35.98	35.26	72.20	75.79
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
Significant Interactions												
Quality groupings*lairage			0.03	NS	NS	NS	NS	NS	NS	NS	NS	NS

$^{a-d}$ Means±SD with different letters are significantly different within a column.

[1]TG (total glucose measured at different times post-mortem and presented as μmol/g of fresh meat); Lac (lactate concentration measured at different times post-mortem and presented as μmol/g of fresh meat).

Table 7-6 Thigh muscle metabolites measured at different times post-mortem for normal or DFD meat from control or cold exposed birds during simulated transport.

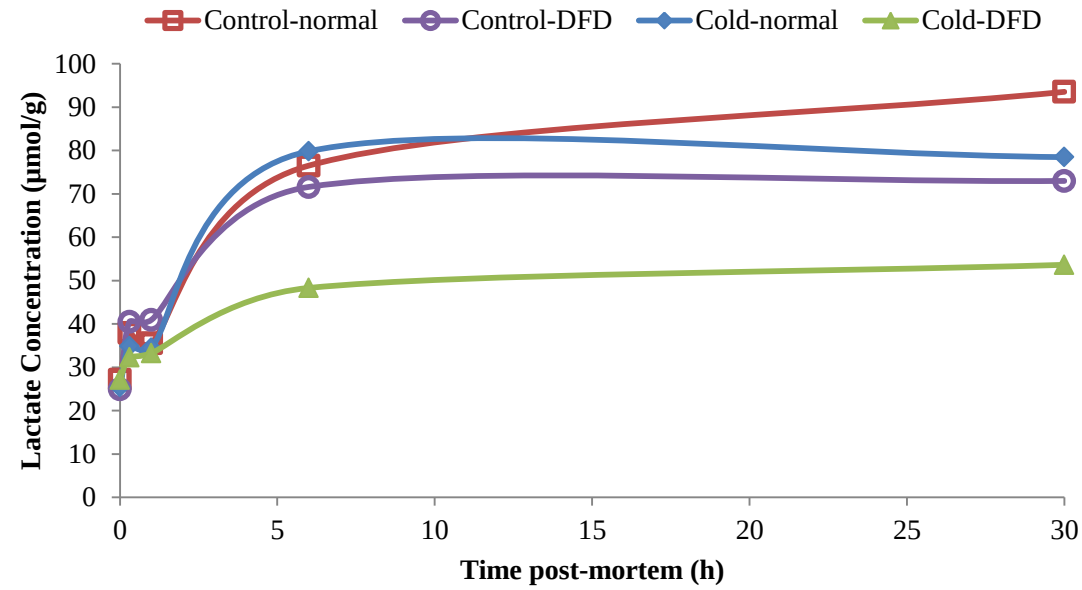
Variables[1]		N	TG $_{5min}$ (μmol/g)	TG $_{1h}$ (μmol/g)	TG $_{30h}$ (μmol/g)	Lac $_{5min}$ (μmol/g)	Lac$_{1h}$ (μmol/g)	Lac $_{30h}$ (μmol/g)	GP $_{5min}$ (μmol/g)	GP $_{1h}$ (μmol/g)	GP $_{30h}$ (μmol/g)
Quality grouping	Control-normal	20	23.4±5.1^{a}	21.5±3.7^{a}	4.1±1.1^{a}	29.6±5.8^{a}	34.9±5.3^{a}	65.6±8.2^{a}	76.4±9.0^{a}	77.8±9.0^{a}	73.8±9.9^{a}
	Cold-normal	3	9.1±5.5^{b}	10.4±7.9^{b}	3.0±1.3^{b}	29.9±8.8^{a}	31.8±5.3^{a}	43.3±16.2^{b}	48.1±18.1^{b}	52.7±19.5^{b}	49.3±16.6^{b}
	Cold-DFD	17	2.4±1.4^{c}	2.2±2.0^{c}	2.4±0.6^{b}	10.1±6.1^{b}	12.5±6.2^{b}	15.3±9.0^{c}	14.8±7.9^{c}	16.9±9.7^{c}	20.0±9.7^{c}
***P*-value**			**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Age (wk)	5	20	13.09	12.19	2.82b	21.97	26.04	30.35b	48.14	50.41	43.99b
	6	20	13.19	12.71	3.70a	20.18	24.30	46.80a	46.56	49.72	54.21a
***P*-value**			**NS**	**NS**	**0.004**	**NS**	**NS**	**0.0215**	**NS**	**NS**	**0.0092**
Lairage (h)	0	20	13.42	12.13	2.96b	18.81a	24.38	38.85b	45.64	48.64	44.76b
	2	20	12.85	12.76	3.56a	23.51b	25.97	46.31a	49.20	51.49	53.43a
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**	**0.0465**	**NS**	**NS**	**0.0304**
Significant Interactions											
Quality grouping*lairage			NS	NS	0.041	NS	NS	NS	NS	NS	NS

[a-d] Means±SD with different letters are significantly different within a column.

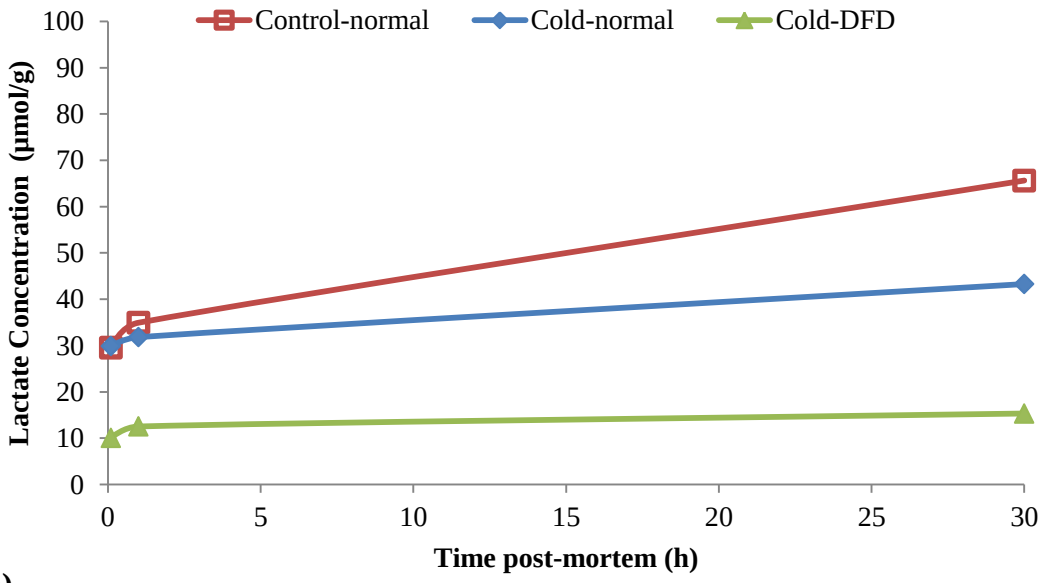
[1]TG (total glucose in μmol/g); Lac (lactate concentration in μmol/g); GP (glycolytic potential calculated based on total glucose and lactate measured at different times post-mortem and presented as μmol of lactate per g of fresh meat.

Breast meat lactate concentration did not show any significant differences up to 6 h post-mortem which agrees with pH data (Figure 7.4). But, by 6 h post-mortem lactate concentration was significantly lower for DFD breast meat from cold stressed birds compared to other groups (Table 7.5; Figure 7.4). However, breast meat with DFD characteristic from both control and cold-stressed birds showed significantly lower lactate content compared to their normal counterparts at 30 h post-mortem, which explains the differences in pH_u and ultimate quality between DFD and normal breast meat due to effect of lactate concentration on meat pH. However, it should be noted that lactate concentration almost doubled for DFD breast meat from the cold stressed birds and tripled for all other samples. Yet, only a very small change in pH of DFD breast meat of cold-stressed birds was observed (by 0.1 unit). Therefore, glycogen at slaughter and lactate concentration at 30 h post-mortem only partially explains acidosis in breast meat. On the other hand, lactate concentration in DFD thigh meat was significantly lower at all times post-mortem compared to normal thigh meat from either control or cold birds, which were only different from each other at 30 h post-mortem (Table 7.6; Figure 7.4).

Glycolytic potential (GP), an indicative of potential lactate produced in the muscle was significantly lower for DFD thigh meat compared to normal thigh meat, which in turn was significantly lower than controls (Table 7.6). The differences in concentrations of post-mortem GP between the DFD and normal thigh meat were more pronounced compared to the breast meat (Figure 7.5). However, DFD breast meat from the cold-stressed birds showed significantly lower GP at all times post-mortem compared to normal breast meat, but not the DFD breast meat from the control birds (Table 7.7). No changes was observed in GP post-mortem for DFD thigh meat, whereas DFD breast meat showed potential energy reserve to carry on post-mortem metabolism and some changes were observed in total glucose, lactate and GP, but of much smaller magnitude compared to the normal breast meat (Figure 7.2-7.5). It is worth noting that breast meat even with extreme DFD properties still has similar GP compared to normal thigh meat and for some reason part of glycogen reserve in breast meat remained unused by the bird during severe cold stress.



(a)



(b)

Figure 7-4 Lactate concentration measured at different times (0 to 30 h) post-mortem in breast (a) and thigh (b) muscles.

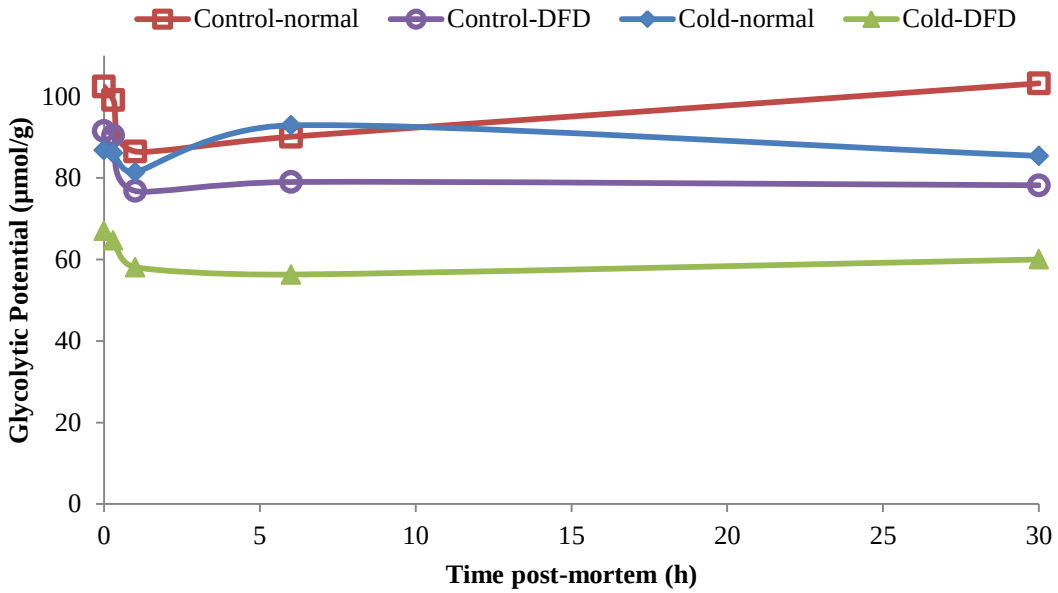
These observations on the differences between breast and thigh meat could be related to the difference in fiber type between the two muscles as discussed earlier, with glycolytic breast muscle not being completely depleted of glycogen even at severe cold exposure conditions, compared to total exhaustion of stored glycogen in oxidative red fibers of the thigh meat from birds exposed to temperatures below 0ºC (Figure 7.5).

Table 7-7 Breast meat glycolytic potential (GP) measured at different times post-mortem for normal and DFD breast meat from control or cold-stressed birds considering age effect and lairage prior to slaughter.

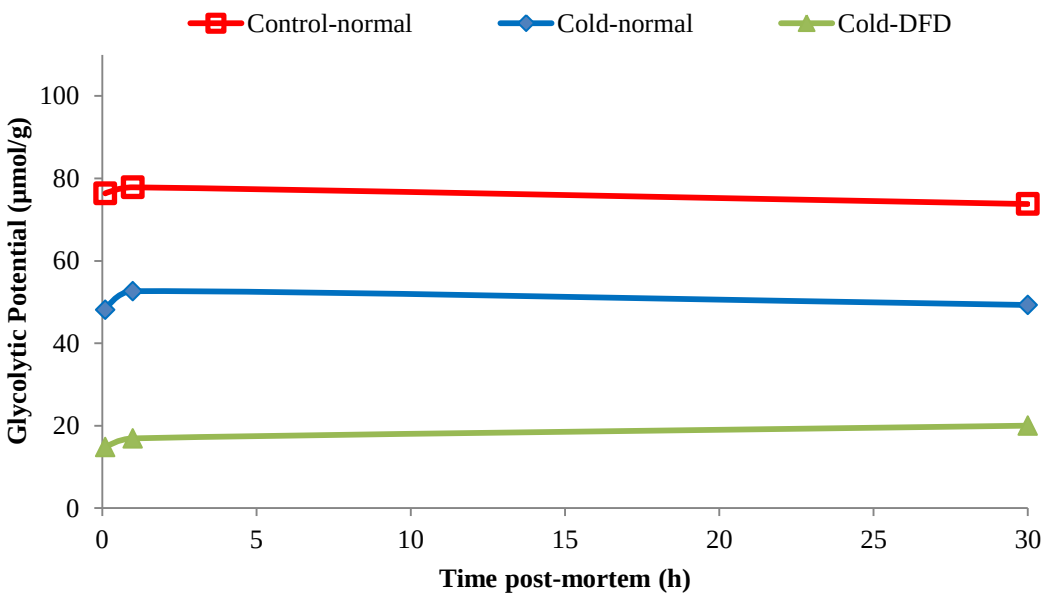
Variables[1]		N	GP $_{0min}$	GP$_{20min}$	GP $_{1h}$	GP $_{6h}$	GP $_{30h}$
Quality groupings	Control-normal	15	102.4±17.0^{a}	99.1±10.6^{a}	86.5±8.5^{a}	90.1±10.0^{a}	103.2±19.9^{a}
	Control-DFD	5	91.5±12.2^{a}	90.4±6.2^{a}	76.8±7.7^{a}	79.0±16.1^{a}	78.2±4.8^{bc}
	Cold-normal	13	86.8±17.6^{a}	86.1±13.9^{a}	81.4±14.2^{a}	92.9±20.8^{a}	85.4±21.5^{ab}
	Cold-DFD	7	67.0±20.6^{b}	64.7±22.4^{b}	58.1±27.4^{b}	56.3±17.6^{b}	60.0±17.2^{c}
***P*-value**			**0.062**	**0.081**	**0.019**	**0.007**	**0.079**
Age (wk)	5	20	93.18	85.10	77.55	80.90	91.43
	6	20	86.32	90.47	79.73	86.56	82.04
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**
Lairage (h)	0	20	89.09	87.83	81.74	84.22	89.49
	2	20	90.41	87.74	75.54	83.24	83.99
***P*-value**			**NS**	**NS**	**NS**	**NS**	**NS**
Significant Interactions							
Quality groupings*lairage			NS	NS	NS	NS	NS

[a-d] Means±SD with different letters are significantly different within a column.

[1]GP (glycolytic potential calculated based on total glucose and lactate concentrations at different times post-mortem and presented as μmol of lactate per g of fresh meat).



(a)



(b)

Figure 7-5 Glycolytic potential (GP) measured at different times (0 to 30 h) post-mortem in breast (a) and thigh (b) muscles.

As glycolytic potential (GP) is measure of potential lactate produced post-mortem and ideally accounts for the various post-mortem metabolites (glucose, glucose 1-phosphate (G1P), glucose 6-phosphate (G6P), and lactate), it was expected to be similar from 0 min to 30 h post-mortem. However, this is only true for thigh meat and not breast meat (Figure 7.5). Thigh muscle GP values measured at different time post-mortem were not different from each other leading to almost a level line as observed in Figure 7.5. However, a slight shift in GP was observed for all thigh samples regardless of quality grouping from 5 min to 1 h post-mortem (Figure 7.5), which slightly decreased thereafter for control samples and slightly increased in thigh meat of cold-stressed birds. The fluctuations observed in breast meat GP calculated at different times post-mortem were more dramatic compared to thigh meat. GP was shown to decrease after 0 min post-mortem in both normal and DFD breast samples, and then increase after 1 h post-mortem for normal breast meat and after 6 h post-mortem for DFD breast meat. GP values for normal breast meat reached the initial (0 min) values by 30 h post-mortem, but remained at lower than 0 min values for DFD samples at 30 h post-mortem (Table 7.7; Figure 7.5). It was speculated that perhaps GP calculation over time did not account for all potential intermediates in the process, which resulted in a drop in GP values within the first 60 min post-mortem. However, by 30 h post-mortem GP values were shifted up to that of 0 h post-mortem for the normal samples, indicating that those potential intermediates were converted to lactate. On the other hand, this trend was not observed for DFD breast samples where ultimate GP values stayed at lower levels compared to the initial GP values (Table 7.7; Figure 7.5), indicating that for some reason, which is not clear at this point the intermediate substances were not completely converted to lactate by 30 h post-mortem.

It was previously reported that in muscles with normal rates of glycolysis, G6P levels tend to decrease during the first h post-mortem and increase thereafter (Hammelman et al., 2003) that might explain the observed fluctuations for the normal samples in the current study. The decrease in G6P is indicative of insufficient G6P supply by glycogen phosphorylase (GPhos) for the subsequent reactions of the glycolytic pathway (Scheffler and Gerrard, 2007), which was speculated to be due to relatively high levels of ATP and low levels of AMP and IMP causing insufficient

activation of GPhos *b*. However, after the first h post-mortem, changes in the relative concentrations of allosteric activators (ATP, AMP and IMP) may permit GPhos activation (Scheffler and Gerrard, 2007). Therefore either the increase in GPhos or a decrease in the activity of phosphofructokinase (PFK) or other enzymes downstream might cause the increase in G6P after 1 h post-mortem. In the case of slow glycolyzing muscles (greater proportion of red fiber) an imbalance between fructose 6-phosphate and fructose 1,6-bisphosphate is reported after 60 min post-mortem, speculated to be due to the decrease in muscle pH and partial inactivation of PFK (ATP is required for transfer of phosphate group to fructose 6-phosphate). PFK was reported to be more likely a glycolytic control compared to pyruvate kinase (Scheffler and Gerrard, 2007). The decrease in G6P during the first 60 min and its subsequent increase thereafter was reported to be indicative of an imbalance between glycogenolysis and glycolysis. Overall, Scheffler and Gerrard (2007) in their comprehensive review suggested that the relative levels of glycolytic regulators contribute to altered post-mortem glycolysis based on cellular energy status, causing different enzymes to be rate limiting at different times during the conversion of muscle to meat.

Maribo et al. (1999) reported that GP can be measured at either 0 or 30 h post-mortem in porcine *m. longissimus dorsi* muscle with a correlation coefficient of 0.83 between the two GP values. However, the correlation between the two GP values measured at 0 and 30 h post-mortem for breast muscle was only 0.52 in the current study compared to 0.96 correlation between GP measured at 5 min and 30 h post-mortem for thigh meat. No report is available on the GP measured at different times post-mortem on either breast or thigh muscles, but the current study showed some time sensitivity for GP measured at different times post-mortem. However, GP time sensitivity was fiber type related; where GP measured for glycolytic breast muscle was time sensitive, but for oxidative thigh muscle similar values were obtained for GP throughout the post-mortem period. Therefore, based on observed values it was suggested that if breast meat GP was measured immediately after slaughter it would be a better representation of energy status at the moment of death. Yet, in regard to meat quality prediction, GP was able to differentiate the normal and DFD breast meat at any time post-mortem, which is the major advantage of using GP over either glycogen or

lactate measured alone. On the other hand, since thigh meat GP was stable at all times post-mortem it could be a very good indicator of thigh meat quality regardless of time of measurement.

Breast muscle temperature was measured at several times post-mortem in order to investigate if there is a relation between muscle temperature at slaughter and development of DFD breast meat. It was assumed that the low temperature of breast meat from cold-stressed birds could be one of the contributors of DFD defect in addition to energy depletion at time of slaughter. It was speculated that low temperature of breast meat at slaughter might have prevented post-mortem metabolism via inactivation of enzymes involved in glycogenolysis or glycolysis. A significant drop in breast meat temperature (~6ºC), measured immediately after defeathering, was observed for birds exposed to temperatures below 0ºC regardless of quality defects (Figure 7.6). Since no significant difference in initial breast meat temperature was observed between the normal and DFD breast meat of the cold-stressed birds, the hypothesis that low temperature of breast meat might be associated with development of DFD defect in broilers was rejected.

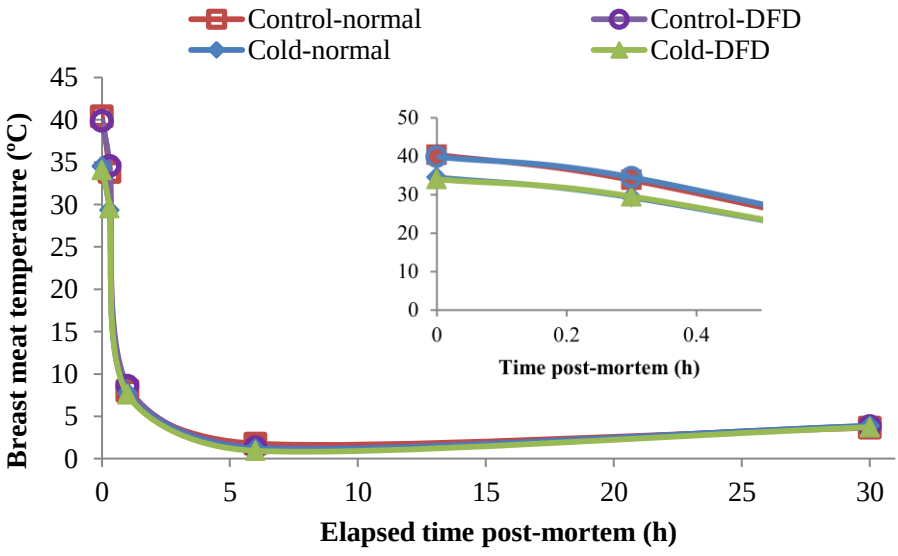


Figure 7-6 Rate of drop in breast meat temperature from 0 to 30 h post-mortem. Inset: 0 to 0.5 h.

Metabolic changes leading to DFD defect is different between breast and thigh muscles. Apart from having different initial energy reserves originally, the rate and extent of metabolism was shown to be different between the two muscles. White, glycolytic breast muscle did not show any difference based on quality grouping in lactate production or pH until 6 h post-mortem, whereas metabolism in red, oxidative thigh muscle was different between quality groupings right after slaughter. Therefore, quality defect in thigh meat could be predicted much better based on post-mortem metabolism compared to breast muscle. Since, DFD thigh meat is completely exhausted of glycogen reserve that explains the lack of change in metabolites post-mortem. But, incidence of DFD breast meat cannot be explained based on glycogen reserve alone. Karlsson et al. (1994) reported that muscle samples with more than 30% depleted IIB fibres (that is similar to fast-twitch) tended to have meat with more DFD characteristics. In the current study, total glucose of DFD meat from cold-stressed and control birds was 47 and 11% lower than total glucose of the normal meat from the control birds. However, normal breast meat from the cold-stressed birds also showed 18% lower total glucose compared to normal breast of control birds. Therefore, the exact reason for occurrence of DFD defect in breast muscle remains unanswered. Even though the total glucose in the breast meat of cold-stressed birds with DFD characteristics was shown to be lower than controls at slaughter, it is not different from the normal thigh muscle. Therefore the question remains as to why thigh meat can use this energy reserve and produce normal meat, whereas breast muscle cannot contribute this energy reserve towards a reduction in pH. Hence in the next section, activity of AMPK in relation to DFD breast meat development was investigated.

7.4.3 Role of AMP-activated protein kinase in post-mortem glycolysis of DFD breast meat

In the preceding section and Chapter V of this thesis it was shown that substrate availability is not the only reason for development of DFD defect in breast meat of broiler chickens. Therefore, lack of activity of the enzymes catalyzing the reactions of glycogenolysis and glycolysis was questioned. These enzymes influence the rate and extent of pH decline by directly controlling the conversion of metabolites through the

glycogenolysis and glycolysis pathways. Major enzymes involved in glycogenolysis are glycogen phophorylase, glycogen debranching enzyme (GDE), PFK and pyruvate kinase (PK). In a comprehensive review by Scheffler and Gerrard (2007) on the rate limiting enzymes involved in PSE pork, it was suggested that GPhos, glycogen debranching enzyme and PFK are not likely to be the main factors involved in altered metabolism in PSE muscle of pork. The involvement of GDE was questioned in development of DFD breast meat in the current study, since the enzyme activity is mainly temperature related (Kyla-Puhju et al., 2005). However, the possibility of this enzyme being involved was very small, since no difference in breast meat temperature was observed between DFD and normal breast meat from either control or cold-stressed birds, with both DFD and normal breast meat of cold-stressed birds showing lower temperatures compared to normal or DFD breast meat of control birds. In addition, it was reported that even temperatures as low as 4ºC is enough for the activity of GDE to produce meat with normal characteristics (Kyla-Puhju et al., 2005). No information is available regarding GDE enzyme activity involved in DFD meat up-to-date. Recently studies have focused on AMPK as a key enzyme in post-mortem metabolism (Shen and Du, 2005a, b; Shen et al., 2005, 2006, 2007; Sibut et al. 2008).

In order to further investigate the DFD defect in breast meat of broiler chickens, given that no difference was observed in energy reserve between normal and DFD breast meat of the control birds, it was hypothesized that lack of AMPK activity might be responsible for cessation of glycolysis and resultant high pH_u of the breast meat. Therefore, several samples were tested for AMPK activity measurement through immunoblotting of the p-AMPK at α-Thr^{172}. The test was performed several times on normal and DFD samples collected at 0 min post-mortem, following the methods proposed by Shen and Du (2005a) for mice and pigs and the proposed method by Sibut et al. (2008) for chickens. In both methods AMPK activity was determined through assessing the concentration of p-AMPK (correlated to its activity) through fluorescent Western Blotting using appropriate antibodies. Concentration of protein was adjusted to enable the comparison of the samples. In addition, vinculin (housekeeping enzyme) was used as a loading control (Figure 7.7). Total AMPK concentration was assessed for all

the samples, where no difference was detected based on quality defects between the samples (Figure 7.8). However, no detectable band was observed for phosphorylated AMPK α-Thr^{172} (expected at about 63 kD). A number of manipulations were performed on lysis buffer, length of electrophoresis, type of primary antibodies, dilution buffers, blocking buffers and other factors that might have affected the detection of the p-AMPK α-Thr^{172}. Yet, the p-AMPK α-Thr^{172} was not detected.

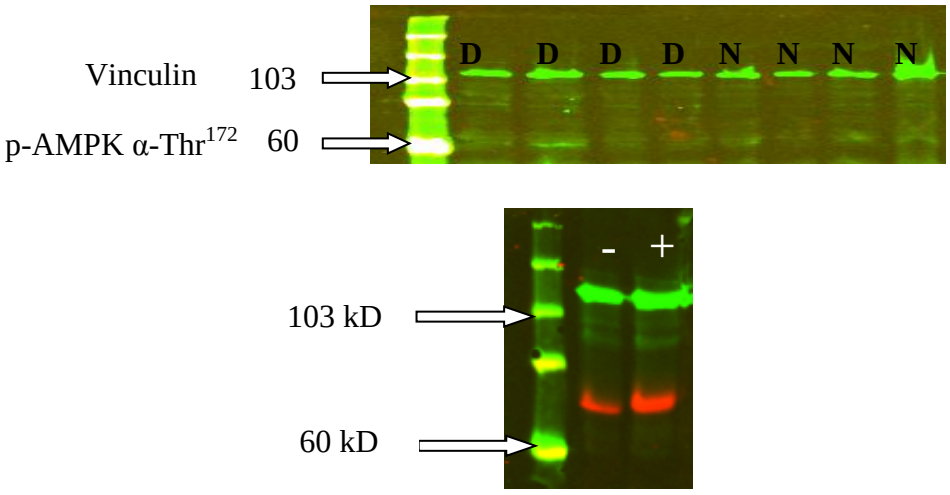


Figure 7-7 Representative Western blots of the Vinculin (used as loading control; 110 kD) and p-AMPK α-Thr^{172} (63 kD), showing the effect of AMPK activity in the *Pectoralis* major muscle of broilers with normal (N) and DFD (D) characteristics (top picture). Positive and negative control for the activity of AMPK (bottom picture).

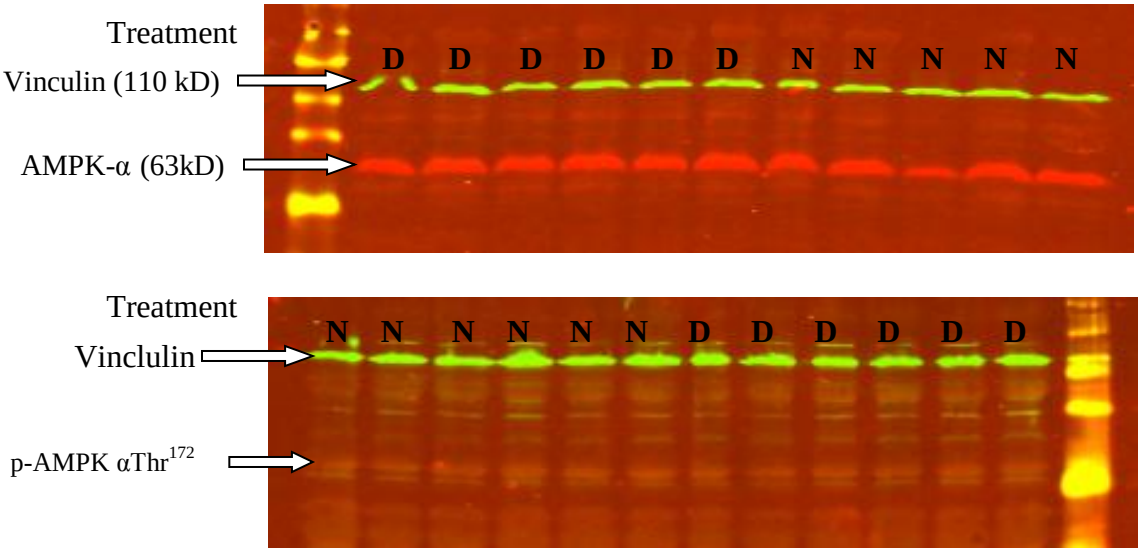


Figure 7-8 Representative Western blots of the AMPK-α (top) and p-AMPK α-Thr^{172} (bottom) for normal (N) and DFD (D) breast meat.

Untreated AMPK control cell extracts from C2C12 cells, prepared with serum served as a negative control, and treated AMPK control cell extracts from C2C12 cells, prepared with serum and hydrogen peroxide treatment (10 mM for 5 minutes), and served as a positive control. Since the positive and negative control bands were detectable (Figure 7.7), and also the total AMPK and vinculin bands were observed, it was assumed that the primary antibody was not responsive for chicken species (not able to detect p-AMPK α-Thr^{172} from chicken); which most probably is not the case, because Sibut et al. (2008) have used the same method for chicken species and have presented results. Another speculation was that the AMPK activity was too low at 0 min post-mortem that it could not be detected with this method. On the other hand, Scheffler and Gerrard (2007) hypothesized that phosphorylation of AMPK might not be the only mechanism by which AMPK modulates energy balance in muscle, therefore further studies are required to support their hypothesis. As a result, difficulties with the methodology did not allow one to draw many conclusions for this part of the study.

Another method for assessing the activity of AMPK is a radioactivity method using a SAMS peptide (His-Met-Arg-Ser-Ala-Met-Ser-Gly-Leu-His-Leu-Val-Lys-Arg-Arg; Invitrogen), which is a synthetic peptide, as a substrate for AMPK activity measurement. In the aforementioned method AMPK activity is calculated as nmol of

ATP incorporated into a SAMS peptide per gram of muscle per minute as described by Winder and Hardie (1996). However, not having access to such facilities did not let us to perform this method. Based on all the studies conducted by Shen and Du (2005 a, b) and Shen et al. (2005, 2006, 2007), one would expect to see lower activity of AMPK for DFD samples compared to normal samples, since higher activity of AMPK was correlated with lower ultimate pH in mice and pigs. However, in the study by Sibut et al. (2008) on broiler chickens, higher activity of AMPK was correlated with higher ultimate pH. Thus, there remains a great deal of contradictory information, which the present study did not resolve.

7.5 Conclusion

Birds exposed to temperatures below freezing developed more DFD thigh (85%) than DFD breast (42%). On the other hand, no DFD incidence for thigh meat was observed within the control birds, compared to 20% DFD breast meat from birds exposed to control temperatures during simulated transportation. Therefore, occurrence of DFD thigh meat was mainly related to cold exposure and depletion of muscle energy reserves, whereas this conclusion cannot be drawn for breast muscle. Initial muscle energy reserves at time of slaughter play an important role in post-mortem metabolism of breast muscle as well, yet there are other factors such as regulatory enzymes or hormonal influences that might have significant effects on the rate and extent of metabolism after slaughter. No significant differences were observed in post-mortem metabolism between the normal and DFD breast meat within the first h after slaughter regardless of temperature treatment (control or cold-stressed). However, rate of pH drop, or lactate production was significantly different after 1 h post-mortem between normal and DFD breast meat from both control and cold-stressed birds. On the other hand, post-mortem metabolism was significantly different between DFD and normal thigh meat from 5 min post-slaughter onward. In addition, exposure temperature and reductions in CBT had larger effects on pH_u of thigh meat compared to breast meat.

It was shown that GP is an important factor for measuring energy status at the moment of death in chicken breast and thigh meat and could be used as a means to differentiate normal and DFD samples at any time post-mortem. However, despite published statements to the contrary, GP was shown to be time sensitive for breast meat but not for thigh meat. Thigh meat quality parameters showed higher correlations with muscle energy reserve at any time post-mortem and pH measured at different times post-mortem compared to breast meat. As a result, quality defect in thigh muscle could be predicted a lot clearer based on energy reserve at slaughter compared to breast muscle. Although, no activity was detected for AMPK in this study, role of this enzyme in post-mortem metabolism of chickens is inconclusive and more work is required to assess its effect in post-mortem glycolysis of broiler breast meat. And perhaps to reveal if there is any relation between breast meat DFD development and activity of AMPK at slaughter in breast muscle. Therefore, measuring the activity of this enzyme and other enzymes involved in glycogenolysis/glycolysis in broiler breast meat might be beneficial in answering the questions raised in regard to breast meat DFD development.

8 GENERAL DISCUSSION

The overall objective of the research leading to this thesis was to improve commercial transportation of birds from farm to the slaughter plant during the winter season by considering vehicle design, bird welfare and subsequent meat quality. To this end, an actively ventilated transport vehicle designed by engineers at the University of Saskatchewan, the "University of Saskatchewan Test Trailer", was employed to transfer birds from the farm to the slaughter plant under cold winter conditions. In order to improve commercial trailers, it is necessary to know the temperature profile throughout the trailer and understand the effect of cold winter transportation on broiler chicken physiological parameters and meat quality attributes. Therefore, 150 Hygrocon iButtons were strategically placed throughout the test trailer to measure temperature and humidity during transport. Birds were individually housed (15 birds/drawer) in order to facilitate the monitoring of the immediate environment surrounding each broiler throughout transportation. Bird physiological status was assessed by measuring core body temperature (CBT). In addition, meat quality parameters including, breast meat pH, color, drip loss, thaw loss, cook loss, shear, water binding capacity (WBC), and processing cook yield (PCY) were assessed.

In the first phase of the study (Chapter III), six transportation trials were conducted at ambient temperatures of -27, -22, -17, -5, +4, and +11ºC, each testing 90 birds (39-42 d old ranging from 2.2-2.3 kg live weight) placed in set locations within the transport vehicle with a capacity of approximately 2900 birds. Birds were indosed with Thermocron iButtons to monitor their internal body temperature during the trial. Transport duration was 3-4 h and lairage duration was 30 min to 120 min, based on arrival time to the slaughter plant. This phase of the study was driven by the rationale that 'cold winter transportation affects bird physiology and subsequent breast meat quality'. The uniqueness of the current research was conducting trials under severe cold

temperatures, representative of Saskatchewan winters, and assessing the correlation between the temperature in birds' immediate surrounding and their CBT with their subsequent breast meat quality attributes. To our knowledge no study had previously looked at cold winter transportation with the approach we employed in this research. However, winter transportation of broilers was previously studied by Hunter et al. (1999) without assessing meat quality and with a minimum transport temperature of -4ºC. Cool ambient temperatures (4, 5 and 7ºC) prior to slaughter were reported to cause a moderate increase in pH_u that resulted in darker color breast meat with better functional properties (Babji et al., 1982; Froning et al., 1978; Holm and Fletcher, 1997). Lee et al. (1976) reported no significant effect of cold exposure (4ºC) or extreme cold exposure (-20ºC) for a duration of 6 h on breast meat tenderness, muscle glycogen level or rate of glycolysis from 8 wk old female broilers.

Birds were taken as experimental units throughout this thesis and grouped based on the experienced temperature during transportation due to the large temperature variation within the transport vehicle in each trial. Birds were designated into four groups of ≤0; 0-10, 10-20 and >20ºC. Birds exposed to temperatures below freezing had a significantly lower CBT compared to other groups. Despite the large differences in the experienced temperature between the groups, the drop in CBT was small (~1ºC). Temperatures below freezing during transport also resulted in darker breast meat with higher pH_u (by 0.1 unit), WBC and PCY. However, no significant difference in the WHC of intact breast meat (drip, thaw and cook losses) or tenderness was observed due to cold winter transport. Higher pH_u (Babji et al., 1982; Holm and Fletcher, 1997) and darker color (Bianchi et al., 2006; Petracci et al., 2004) for poultry breast meat due to exposure to cool temperatures prior to slaughter was previously reported. The incidence of DFD (pH>6.1, L*<46) in breast meat was observed only with exposure temperatures below 0ºC (8%), with an overall frequency of about 1.5% DFD incidence. On the other hand, incidence of PSE was significantly ($P < 0.05$) higher at temperatures above 20ºC (13%) compared with temperatures below 0ºC (4%). This finding agrees with several other studies that reported higher incidence of PSE breast meat in turkey and chicken as a result of seasonal heat-stress (McKee and Sams, 1997; Owens and Sams, 2000;

Petracci et al., 2004) and reduced occurrence (5.9%) of PSE breast meat in birds processed during winter (Bianchi et al., 2006) and increased breast meat with DFD characteristics during winter transportation (Lesiów et al., 2007; Petracci et al., 2004).

Accomplishment of the first study raised a lot of questions, leading to the design of the second phase of this research study where an environmental chamber was created to simulate the transport conditions. This simulated transport chamber allowed more control over the transport situation and enabled assessment of physiological parameters, such as blood metabolites and live shrink, and close monitoring of pre and post-slaughter steps. In addition, rest time prior to slaughter (lairage), and selection of birds based on age and gender enabled assessment of these variables during simulated cold transportation on subsequent meat quality. Based on the results of the field trials, it was hypothesized that 'severe cold temperature will increase incidence of DFD breast meat and age and gender of birds, as well as lairage prior to slaughter, might affect bird survival during severe cold conditions and subsequent meat quality'.

As discussed in Chapter IV, different cold temperatures ranging from -18 to -4ºC were employed along with a control of +20ºC for duration of 3 h on 360 broilers equally selected for gender and age within 5 (35-37 d) or 6 (40-42 d) wk old birds. Similar to the first part of this study, birds were individually housed and CBT as well as immediate surrounding temperatures were monitored by the minute. Birds were assigned to 0 or 2 h of rest after the trial and prior to being hand slaughtered in the slaughter abattoir. Meat samples were collected at 5 min and 30 h post-mortem for analysis of breast muscle metabolites. Meat quality attributes similar to first phase were assessed and birds were assigned into 5 groups: $-17<T\leq-14$, $-14<T\leq-11$, $-11<T\leq-8$, $-8<T\leq0$ and $20<T\leq22$. A substantial (5 to 15ºC) drop in CBT of birds was observed as their immediate temperature dropped below -8 and -14ºC based on age (5 or 6 wk old, respectively). Twenty three percent of 5 wk birds suffered from severe hypothermia (their CBT dropped below 20ºC) when exposed to temperatures below -14ºC compared to only one among the 6 wk old birds. Therefore, 6 wk birds (2.6 kg) could better manage their CBT during the 3 h of simulated transport under severe cold temperatures compared to 5 wk birds (1.9 kg), which was related to the larger body size and more

feather coverage of 6 wk birds. Males (2.5 kg) had a greater drop in CBT (by 0.3ºC) compared to females (2.1 kg), which was associated with more feather coverage and higher abdominal fat for females compared to males. The 2 h of lairage prior to slaughter exacerbated the situation for some birds, depending on the physiological status of birds at initiation of lairage. However, the majority of birds managed to recover to their normal CBT by the end of lairage.

The drop in CBT observed in this phase of the study (Chapter IV) was much greater than in the actual transport study, which was related to the lower experienced temperature of the birds and differences in age. However, when comparing only the 6 wk birds, results of both studies are similar. The only research studying broiler body temperature in relation to cold winter transport (-4 to +12ºC) was the study by Hunter et al. (1999), which showed dramatic drop of 14.2 ± 5.5ºC in rectal temperature of birds exposed to -4ºC under wet condition. These dramatic drops were only observed at temperatures below -14ºC in this part of our study, which could be explained by dry condition tested here compared to wet condition tested in the Hunter et al. (1999) study.

Live shrink was significantly higher for all the cold temperatures tested (~4%) compared to the control temperature (1.4%), which might be related to shivering thermogenesis and excess energy usage during cold exposure in order to maintain homeostasis. An increase of 0.3% in live shrink was observed with 2 h lairage, which was associated with extended feed withdrawal and usage of energy through catabolising body tissue in order to recover to a normal CBT. Blood glucose concentration showed an incremental decrease as the immediate temperature in the birds' surroundings declined from 0 to below -14ºC, with controls having significantly higher blood glucose concentrations compared to all cold-stressed birds.

Breast meat of birds exposed to temperatures below -14ºC showed lower glycolytic potential (GP) at 30 h post-mortem, regardless of age or lairage. No difference in GP was observed between other temperature groupings. Therefore, at temperatures above -14ºC birds were still able to compensate for cold exposure and maintain adequate reserves of glycogen. Below this temperature, birds were no longer able to compensate for the energy expenditure required to combat the extreme cold

temperatures and a significant drop in muscle GP was observed. In contrast, Lee et al. (1976) did not find any effect of 6 h exposure to -20ºC on breast muscle glycogen of 8 wk broilers. In the present study males also showed lower GP compared to females, which was related to the lower CBT observed for this gender compared to females.

Age of birds showed a significant effect on the majority of meat quality parameters due to cold exposure prior to slaughter. Breast meat from 5 wk birds showed darker and redder breast meat with higher pH_u, lower cook loss and higher PCY at temperatures below -8ºC compared to warmer temperatures and compared to that from 6 wk birds exposed to similar temperatures. Meat from 6 wk birds had higher pH and darker color at temperatures below -11 and -14 ºC, respectively. No difference in meat quality was observed between the two ages at temperatures below -14ºC. The greater impact of cold temperatures ranging from -8 to -14ºC prior to slaughter on 5 wk birds is probably due to the greater difficulties that young birds experienced in coping with the cold conditions due to their greater surface area and less feather cover than older birds.

Effect of age at slaughter on meat quality was mostly temperature dependent as discussed earlier (Chapter IV) and could not be compared to the other studies conducted on different ages (Anadon, 2002; Sandercock et al., 2002). In addition, gender of birds showed some effect on breast meat quality, with females having redder and yellower breast meat with lower pH_u, WBC and PCY, but no difference in L* (lightness) compared to males. A number of studies have looked at the effect of gender on breast meat quality (Anadon, 2002; Berri et al., 2007; Ngoka et al., 1982), where findings of Anadon (2002) are in agreement with this phase of our study. The 2 h of lairage following simulated transport resulted in significantly ($P < 0.05$) darker and less yellow (3.9 vs. 4.3) breast meat with lower cook loss and thaw loss compared to meat of birds slaughtered without lairage. The effect of lairage on pH, WBC and PCY was mainly observed when birds were exposed to extremely cold temperatures (<-11 and -14ºC for pH and WBC respectively) prior to slaughter. Therefore, lairage effect was also temperature dependent and was affected by status of birds at the initiation of lairage.

Experienced temperature and CBT during simulated or actual transportation only partially explained meat quality attributes, as the correlations were very low (r ~

0.4). Glycolytic potential correlated well ($P < 0.0001$) with meat quality parameters, including pH_u, color L* and b*, cook loss, WBC and PCY, predicting over 50% of the variation in pH_u. GP was considered as a good measure of meat quality attributes, which agrees with previously reported studies (Soares et al., 2007; Van Laack et al., 2000).

In order to compare the first two phases of this study (Chapter III and IV) on the effect of cold transportation on meat quality, only 6 wk old birds from the simulated transport study were compared to the birds exposed to temperatures below 0ºC during actual transportation. In both studies, exposure of birds to temperatures below freezing during actual or simulated transport resulted in significantly darker and redder breast meat with higher WBC and PCY. In the actual transportation trial, birds exposed to temperatures below 0ºC had significantly higher pH_u, but in the simulated transport trial pH_u was higher when temperature dropped below -11ºC. Yet, it should be kept in mind that overall pH was higher in the simulated transport study compared to the actual transport study, where birds exposed to temperature between 20 to 30ºC in actual transport trial had pH_u of 5.84 compared to 6.04 for the controls (20 to 22ºC) in the simulated transport trial. In addition, no significant effect of actual transport was observed on WHC (drip, thaw and cook losses), whereas drip and cook loss were significantly lower for birds exposed to temperatures below 0ºC compared to the controls in simulated transport trial. These observed differences between the two studies in quality attributes of breast meat are most probably related to differences observed in breast meat pH, which could be related to biological variability and, as stated by Petracci et al. (2004), pH and color might be different between birds from different flocks. Nevertheless, the higher experienced temperature (25ºC) in actual transportation might partly explain the lower breast meat ultimate pH compared to simulated transport (21ºC).

The incidence of DFD breast meat was very high in this phase of the study (Chapter IV), with 5 wk birds showing 3, 8, 45, 71, 58% and 6 wk showing 0, 7, 9, 24 and 59% incidence of DFD when exposed to temperatures of control, 0 to -8, -8 to -11, -11 to -14 and below -14ºC, respectively. In addition, incidence of DFD was up to 20% higher with 2 h lairage following exposure to temperatures below -8ºC. A similar

incidence of DFD was reported for 39 to 42 d (6 wk) birds following the transportation runs in the first phase of this study after exposure to a similar temperature range during transportation (8% DFD at temperatures below 0ºC). Therefore, the chamber used in the second part of our study was able to simulate cold transportation reasonably well and is a valuable tool for the study of physiological and meat quality parameters in broiler chickens.

As observed in the preceding work, DFD incidence was significantly higher when birds were exposed to extreme cold temperatures during simulated transport and GP was only different at extremely cold temperatures (<-14ºC), which does not explain the differences observed in meat quality and DFD incidence between other groups. It was further shown that cold-induced DFD defect could also be affected by the age of birds and lairage duration prior to slaughter. Several studies have reported that lack of glycogen at slaughter results in high ultimate pH and meat with DFD characteristics in pigs and cows. Therefore the hypothesis that 'cold-induced DFD breast meat occurs due to insufficient energy reserves at slaughter' was tested in the next phase.

For the third phase of the study (Chapter V), further research was carried out on the samples collected from birds studied in the second phase (Chapter IV). The effect of liver glycogen at time of slaughter and muscle energy reserves at 5 min and 30 h post-mortem on the incidence of DFD breast meat was evaluated. Breast samples were categorized based on the pH_u and color L* into normal ($5.7 \leq pH_u \leq 6.1$; $46 \leq L^* \leq 53$) breast meat from control (+20ºC) or cold-stressed (0 to -17ºC) and DFD ($pH_u > 6.1$; $L^* < 46$) breast meat from the cold-stressed birds. No DFD defect was observed within breast meat of the control birds, which agrees with the first phase of this study that no DFD defect was observed at transport temperatures above 0ºC.

The average temperature that the birds experienced was significantly lower for cold-stressed birds with DFD defect (-13.6ºC) compared to their normal counterparts (-9.3ºC). In addition, birds with DFD breast meat showed significantly ($P < 0.05$) lower CBT (37.6ºC) compared to their normal counterparts (38.8ºC) and controls (40.6ºC), which is similar to what was observed in the actual transportation in the first phase of this study. Low levels of blood glucose in birds with DFD breast meat compared to

normal breast meat could be a predictor of DFD development in breast meat, or better to say drop in blood glucose below 8 mmol/L could induce development of DFD breast meat in broilers. Low levels of glycogen in the liver (15 μmol/g) could have also initiated the development of DFD breast meat in the cold-stressed birds compared to birds with normal breast meat (20 μmol/g).

Breast meat with DFD characteristics had significantly ($P < 0.05$) higher pH_u (6.4 vs. 6.1), lower L* value (42 vs. 48), lower cook loss (10% vs. 13%), higher WBC (49% vs. 39%) and PCY (122% vs. 102%), but no difference in tenderness compared to normal breast meat, which was in agreement with first phase of the study (Chapter III). No discrepancy between the actual and simulated transport studies was observed based on characteristics or incidence of DFD breast meat. Some significant effects of age and lairage were observed on the incidence of DFD breast meat in broilers. Five wk birds showed higher incidence of DFD breast meat regardless of gender compared to 6 wk birds. This higher incidence of DFD in younger birds is probably associated with lower CBT because no difference was observed based on age in muscle energy reserves. The 2 h lairage period following cold exposure increased the incidence of DFD breast meat, particularly for females of 5 wk old and males of 6 wk old broilers.

The significance of the third phase of this study (Chapter III) was that no differences were observed in initial glycogen or Glucose/G6P concentrations between the cold-DFD and control-normal breast meat. This observation disapproved the hypothesis that cold-induced DFD breast meat is a result of depletion in muscle glycogen at slaughter. Lactate concentration was significantly ($P < 0.05$) lower in meat from the cold-stressed birds, regardless of quality defect, compared to the controls. However, GP was significantly lower for DFD breast meat compared to the normal breast meat from cold-stressed and control birds at 5 min post-mortem. This showed that the ability of DFD breast meat to produce lactate was lower compared to normal breast meat. Initial pH of DFD breast meat was not different from the normal breast meat of the cold-stressed birds, but was significantly lower than that of control birds, showing dependence of initial pH on initial lactate concentration rather than glycogen reserves.

DFD breast meat had a significantly higher pH_u compared to the normal breast meat of both control and cold-stressed birds, which were not different from each other. Breast meat DFD defect was predictable based on GP at slaughter and 30 h post-mortem. As well, concentration of lactate generated by 30 h post-mortem almost tripled for all the samples, regardless of quality grouping, but was significantly lower at 30 h post-mortem for DFD breast meat compared to normal breast meat of cold-stressed and control birds. It is very interesting to note that this increase in lactate concentration caused a reduction in pH of the normal breast meat by 0.22 and 0. 30 for control and cold stressed birds, respectively, whereas it did not cause any changes in the pH of DFD breast meat. Although lactate concentration was lower at 30 h post-mortem in DFD breast samples compared to normal breast, it does not explain the lack of change in pH of the DFD breast meat. According to Puolanne et al. (2002), 10-20 mmol/kg lactic acid derived from 5-10 mmol of glucose equivalents is required to make a change of 0.2-0.3 units in pH. Therefore, one would assume that there would be a change in the pH of DFD breast meat (of ~0.6) and perhaps a bigger change in pH of the normal breast meat (of ~ 0.6-0.8). On the other hand, the difference in lactate concentration between DFD and normal samples is 18-23 μmol/g, which could explain the 0.35 unit difference between pH_u of DFD and normal samples. For this reason, neither initial glycogen nor initial lactate alone were able to predict the ultimate quality of broiler breast meat, while, GP measured at either 5 min or 30 h post-mortem or lactate measured at 30 h post-mortem was able to predict quality attributes of broiler breast meat. Development of DFD meat in broilers remains unexplained because there was enough energy reserve at slaughter and this energy reserve was converted to lactate. Bendall (1973) showed that pH decline can stop, even in the presence of high residual glycogen content, but the reason for this phenomenon was not clearly understood. Scopes (1971) reported that inactivation of glycogenolytic and glycolytic enzymes resulting from disappearance of AMP due to deamination of AMP into IMP could explain the stop of pH decline, even in the presence of glycogen.

The fourth phase of this research study (Chapter VI) investigated the effect of cold-stress during transport on broiler thigh meat quality and its comparison with breast

meat quality with the rationality that 'thigh muscle might respond differently to cold-stress compared to breast muscle'. For this phase of the study, 160 male broilers at two ages of 5 and 6 wk were assigned to cold temperatures of -9, -12 and -15ºC and control of +20ºC in order to induce DFD defect in breast meat using the simulated transport apparatus used for the second phase of this study under the same conditions. Again, birds were given 0 or 2 h of lairage prior to slaughter. Physiological parameters and breast and thigh meat quality attributes were measured.

Birds were grouped into four groups of 0 to -8, -8 to -11, -11 to -14ºC and a control of +20ºC. Similar to second phase of this study (Chapter IV), a significant drop in CBT and blood glucose and an increase in live shrink was observed as a result of exposure to temperatures below freezing, which worsened as temperatures during simulated transport further decreased below -8 and -11ºC. Five wk birds (2.1 kg) showed lower average CBT compared to the 6 wk birds. In addition, 50% of the 5 wk birds showed CBT below 24ºC compared to none of the 6 wk birds (2.9 kg) when exposed to temperatures below -11ºC. This finding was previously observed in the second phase of this study but was of a smaller magnitude, being only ~9% for 5 wk birds exposed to temperatures below -11ºC.

Birds showed lower GP for thigh meat as the exposure temperature decreased from 0 to -14ºC. These differences in muscle metabolites between groupings were very pronounced for thigh compared to breast, showing a greater effect of temperature during transport on thigh muscle metabolites. In addition, a greater effect of environmental temperatures below freezing was observed on thigh pH compared to breast pH, with thigh pH being more greatly (by 0.8 unit) affected compared to breast meat (by 0.1 unit) based on temperature groupings. Furthermore, experienced temperature during simulated transport showed much higher correlations with thigh muscle GP ($r = 0.91$) and meat pH ($r = -0.93$) and color lightness ($r = 0.85$) compared to breast muscle GP ($r = 0.27$), meat pH ($r = -0.36$) and color lightness ($r = 0.29$). Therefore, the temperatures experienced by birds had a more profound effect on thigh meat quality parameters compared to that of breast meat. Muscle temperature measured immediately following slaughter showed a significant drop of up to 12ºC for breast and thigh meat when birds were exposed to temperatures below -11ºC. There was no difference in temperature

between breast and thigh muscles, therefore, these differences in quality parameters might not be explained by muscle temperature at slaughter. Breast meat may also be more tolerant to changes in temperature when compared to thigh meat.

Differences in cold response between muscles could also be related to differences in the role each muscle plays in the body and type of fibers they contain, with the breast *Pectoralis* major muscle being almost completely composed of glycolytic white fibers and thigh *Iliotibialis* muscle being partly composed of oxidative red fibers (Barbut et al., 2002). Several studies have reported a greater effect of transport stress (Debut et al., 2003), feed deprivation, and transport duration (Warriss et al. 1993) on thigh compared to breast meat, which agrees with the present study. In addition, leg muscles are involved in maintaining balance in the moving vehicle during transport, which was speculated to be one of the reasons for differences in response to transport stress between various muscles (Warriss et al., 1993).

In the last phase of this study (Chapter VII), incidence of cold-induced DFD breast and thigh meat was assessed and post-mortem biochemical changes in DFD breast and thigh meat was investigated to find the potential causes for development of this defect. The rationale behind this part of study was that 'breast and thigh muscles might be different in the basis and incidence of DFD development'. In addition, activity of AMP-activated protein kinase (AMPK) was assessed in breast meat samples with the hypothesis that 'lack of activity of AMPK might be causative of DFD development in broiler breast meat'. Muscle metabolites and pH were assessed at different times post-mortem on 40 breast and thigh samples selected within the population of 160 male broilers from the fourth phase of this study. A number of 20 birds from the control temperature (+20ºC) and 20 birds from cold conditions (-9 to -15ºC) was equally chosen within 5 and 6 wk old and similarly assigned to 0 or 2 h of lairage. Post-mortem metabolism was monitored by measuring total glucose, lactate concentration and pH at 0, 20 min, 1 h, 6 h and 30 h post-mortem on breast meat and at 5 min, 1 h and 30 h post-mortem on thigh meat. GP was calculated based on total glucose and lactate concentration at different times post-mortem, and activity of AMPK was assessed by immunoblotting of p-AMPK at αThr^{172}.

Samples were grouped to normal breast ($5.7 \leq pHu \leq 6.1$; $46 \leq L^* \leq 53$) and thigh ($5.9 \leq pHu \leq 6.4$; $44 \leq L^* \leq 51$), or DFD breast ($pH_u > 6.1$; $L^* < 46$) and thigh ($pH_u > 6.4$; $L^* < 44$) meat from control or cold-stressed birds. None of the thigh meat developed DFD characteristics under the control temperature, but some of the breast samples showed meat with DFD characteristics within the control birds, which was in contrast to phase III (Chapter V) of this study. Within the cold-stressed birds, 85% of the thigh samples had DFD properties compared to 42% for breast meat samples, while these values were 0 and 20% for thigh and breast meat of the control birds, respectively. Based on all the experiments conducted on the incidence of DFD breast meat in this thesis, it can be stated that the environmental chamber simulated cold transportation reasonably well. However, the high incidence of DFD at exposure temperatures below -11°C found in the present study has not been reported previously. This may be related to the individual housing of birds during treatment and their inability to huddle together in order to maintain their CBT at acceptable levels throughout the cold exposure. On the other hand, commercial broiler transportation during Canadian winters can present exposure to temperatures below -11ºC (Chapter III). But the ability of birds to huddle together to conserve heat could result in a more moderate situation for broilers in transit. In addition, due to the large variation in temperatures within a transport trailer, only a small proportion of the birds may experience the cold temperatures tested in this study, thus DFD incidence for the whole vehicle would be relatively low.

DFD thigh meat was significantly darker, redder and less yellow, and showed significantly higher pH at any time (5 min, 1 h and 24 h) post-mortem compared to normal thigh meat. As previously reported (phase III; Chapter V), DFD breast meat from the cold-stressed birds had a significantly ($P < 0.05$) darker, redder and less yellow color and higher WBC and PCY compared to the normal breast meat from both control and cold-stressed birds. No pH differences were observed until 6 h post-mortem between the DFD and normal breast meat.

Energy reserves were completely exhausted (2 μmol/g) in DFD thigh meat, suggesting that lack of substrate availability resulted in DFD in thigh meat, whereas in white glycolytic breast muscle total glucose at slaughter was only limited in cold-stressed birds with DFD meat (20 μmol/g) and not in the control birds with DFD breast

meat (33 μmol/g). No change in glucose concentration of *Iliotibialis* muscle of DFD thigh meat was observed and, as a result, pH of this muscle remained unchanged. Total glucose of breast *Pectoralis* major muscle dropped to nil amounts (3 μmol/g) regardless of quality groupings by 30 h post-mortem, which resulted in lactate build-up to double or triple their initial values at death. This increase in lactate concentration did not contribute to a decrease in pH of the cold-DFD breast meat, but pH of the normal-DFD breast meat dropped 0.5 units by 30 h post-mortem. Therefore, as highlighted in phase III of this study (Chapter V), the build-up in lactate did not contribute to a drop in the pH of DFD breast meat from the cold-stressed birds. However, the drop in pH of control-DFD breast meat was not enough to produce normal quality meat by the defined criteria of this study. It should be noted that the degree of DFD characteristics was greater in cold-stressed compared to control birds, and the differences between DFD and normal meat from the control birds might not be even be distinguished by consumers.

Total glucose, lactate concentration or pH measured up to 1 h post-mortem did not seem to be a good predictor of breast meat quality, but they were good measures of thigh meat quality. These differences between breast and thigh meat could be related to the difference in fiber type between the two muscles. Since, glycolytic breast muscle was not completely depleted of glycogen even at severe cold exposure conditions, compared to total exhaustion of stored glycogen in oxidative muscle of the thigh meat from cold-stressed birds. Therefore, some aspects of DFD development are different between breast and thigh meat.

GP was more highly correlated to changes in meat quality of thigh meat rather than breast meat. GP for breast meat was time sensitive and showed some fluctuations over time, but not for thigh meat. It was speculated that perhaps GP calculation over time did not account for all potential intermediates in the process, which resulted in a drop in GP values within the first 60 min post-mortem and then a return to initial values thereafter for the normal meat, and GP of DFD breast meat remained below initial values. This fluctuation in GP may be related to imbalances between glycogenolysis and glycolysis as reported in the study by Hammelman et al. (2003) and the review by Scheffler and Gerrard (2007). Hence, exact reasons for occurrence of DFD defect in

breast muscle remains puzzling. Therefore activity of AMPK in relation to DFD breast meat development was assessed, since an extensive body of literature had shown that AMPK is important for maintaining the activity of GPhos and pyruvate kinase, and glycogenolysis/glycolysis in post-mortem muscle (Shen and Du, 2005a, b; Shen et al., 2005, 2006, 2007; Sibut et al. 2008). Based on these studies, AMPK has a key role in controlling post-mortem glycolysis and its activity is crucial for a lower pH_u in post-mortem muscle (Shen and Du, 2005a).

The total amount of AMPK was similar for normal and DFD breast samples in this study, but p-AMPK could not be detected, leading to an unclear role of AMPK in the development of DFD breast meat. Inability to detect AMPK activity was speculated to be either due to very low activity of this enzyme that could not be detected with immunoblotting of AMPK at α-Thr^{172} or to the inability of the primary antibody to detect p-AMPK α-Thr^{172} from chicken. Scheffler and Gerrard (2007) hypothesized that phosphorylation of AMPK might not be the only mechanism by which AMPK modulates energy balance in muscle, therefore, further studies are required to support their hypothesis. As a result, difficulties with the methodology did not allow one to draw any conclusion about the role of AMPK.

9 GENERAL CONCLUSIONS

Transportation of birds from farm to the slaughter plant (3-4 h) using the University of Saskatchewan test trailer (Chapter III) showed that the immediate temperature surrounding broiler chickens during transportation may affect physiological responses and subsequent breast meat quality by causing variations in breast meat color, ultimate pH (pH_u) and water binding capacity (WBC). Birds exposed to temperatures below freezing showed lower core body temperatures (CBT) and breast meat with higher pH_u, darker and redder color and higher WBC and processing cook yield (PCY), but the effect of transportation temperature on the water holding capacity of intact breast meat was not significant. A higher occurrence of PSE meat was observed as temperatures surrounding the broiler chickens during transportation increased from sub-zero temperatures to above 20ºC. Furthermore, an 8% incidence of DFD breast meat was observed for birds exposed to temperatures below 0ºC during transportation. DFD breast meat was associated with a significantly darker color, with a higher pH_u and a higher water holding capacity compared with normal breast meat. Although significant, the exposure temperature during transportation showed low correlation with meat quality parameters and CBT. Breast meat quality parameters showed very low correlation with CBT of birds during transport.

In the second study (Chapter IV) transportation of birds was simulated in an environmental chamber under extreme cold conditions for 3 h prior to slaughter, considering the effect of bird age, gender and lairage duration prior to slaughter. Birds at 6 wk of age (heavier with more feather coverage) coped better with extreme cold conditions as their meat quality and physiological parameters (CBT, blood glucose, and live shrink) were significantly compromised when temperature dropped below -14ºC compared to -8ºC for the 5 wk birds. As a result, 5 wk birds had breast meat with higher pH_u, darker and redder color, lower cook loss, and higher processing cook yield compared to 6 wk birds. Females coped better with cold exposure by maintaining a higher CBT compared to males, which resulted in breast meat with lower pH_u and WHC for females compared to males. Glycolytic potential (GP) was only affected when exposure temperatures dropped below -14ºC and was shown to be a good predictor of

meat quality parameters by explaining over 50% of the variation in pH_u of the muscle, which in turn is highly correlated with other quality parameters of breast meat in broiler chickens. The incidence of DFD breast meat increased to over 50% for the breast meat of birds exposed to cold conditions (T < -11ºC) in individually housed birds during 3 h of simulated transport. In addition, two hours of lairage following extreme cold exposure caused a further increase of 20% in the incidence of DFD breast meat at temperatures below -11ºC. This study shows that it is possible to induce DFD in broilers by cold exposure prior to slaughter, with cold-induced DFD incidence further influenced by age and gender of the birds and length of lairage.

In the third phase of this study (Chapter V), characteristics of DFD breast meat was further investigated. DFD breast meat showed significantly higher pH_u, darker color and higher processing characteristics, which might be beneficial for further processed products but is a defect for the fresh meat industry due to its dark color. Birds with DFD breast meat had experienced significantly lower temperatures during simulated transport and had lower CBT, blood glucose and liver glycogen compared to birds with normal breast meat either from controls or cold-stressed birds. Muscle glycogen reserves at slaughter was not related to the occurrence of DFD breast meat, showing that glycogen reserves at slaughter may not be a good predictor for quality attributes of broiler breast meat. However, glycolytic potential (GP), that is representative of potential lactate produced post-mortem, was significantly different at slaughter and 30 h post-mortem between the DFD and normal breast meat from both cold-stressed and controls, which could partly explain the differences in pH and meat quality. Lack of change in DFD breast meat pH post-mortem was not explained by the lactate produced during the early post-mortem period (up to 30 h). On the other hand, GP measured at 5 min and 30 h post-mortem correlated well with meat pH_u (0.72) and all of the quality parameters, showing that it could be a valid predictor of meat quality attributes, but it cannot fully explain the variation in broiler breast meat quality. Therefore, questions about DFD development in broiler breast meat remained unanswered.

In the next study (Chapter VI), the effect of cold stress was investigated on quality of broiler thigh meat using the simulated transport system and results were compared to breast meat. Cold-stress showed a greater effect on thigh meat compared to breast meat of broilers during transportation. Thigh meat GP was over 5 times lower for extreme cold stressed birds (T < -8ºC) compared to the control birds, whereas this difference in GP based on exposure temperature prior to slaughter was of a much smaller magnitude for breast meat. The pH_u of thigh meat was different by 0.8 units between control and extreme cold-stressed birds (T < -8ºC), compared to a difference of 0.2 unit in pH of breast meat. Thigh muscle (slow-twitch) was shown to be more prone to glycogen depletion compared to breast muscle (fast-twitch) due to differences in fiber type and type of stress (cold-stress) prior to slaughter. Muscle metabolites showed higher correlations with thigh meat quality attributes compared to breast meat. In addition, exposure temperatures during simulated transport and CBT of birds during transport showed much higher correlations with thigh meat color and pH compared to breast meat color and pH, indicating that exposure temperature and CBT could better explain changes in thigh meat quality.

In the last phase of this study (Chapter VII), the biochemical basis of the cold-induced DFD defect in breast and thigh meat was investigated. Thigh meat was shown to be more prone to development of the DFD defect (85%) compared to breast meat (42%) as a result of cold-stress during simulated transport. Occurrence of DFD thigh meat was mainly related to cold exposure and depletion of muscle energy reserves at slaughter, whereas this conclusion cannot be drawn for breast muscle. Initial muscle energy reserves at time of slaughter were lower in DFD breast muscle of cold-stressed birds, but it was fully converted to lactate. This lactate did not contribute to pH drop of DFD breast meat, therefore, it would be very interesting to further investigate why this build-up in lactate did not cause any drop in pH of DFD breast meat. GP was shown to be time sensitive for breast meat but not for thigh meat, because some fluctuations were observed between GP calculated at different times post-mortem that was contrary to the reported statement that GP is not time sensitive for *longissimus dorsi* muscle of pigs. GP could completely predict thigh meat quality at any time post-mortem; however, for

breast meat, GP could best predict energy status at time of death when measured at 0 min post-mortem. As a result, concentrations of metabolites post-mortem correlated much better with pH of oxidative thigh muscle than glycolytic breast muscle. The role of AMPK in post-mortem metabolism of chickens was inconclusive and more work is required to assess its effect in post-mortem glycolysis of broiler breast meat and reveal if there is any relation between breast meat DFD development and activity of this enzyme at slaughter.

In conclusion, we observed that transportation under severe cold temperatures (similar to Saskatchewan winters) could affect bird physiological aspects, such as CBT and blood glucose content and subsequent breast and thigh meat quality. It was shown that broiler chickens are amazing creatures in combating severe cold temperatures by their ability to survive the harsh transport situation despite a dramatic drop in CBT. This ability of birds to tolerate severe cold conditions was significantly affected by their age at slaughter, with older birds (bigger with more feather coverage) being able to survive better compared to younger birds. In addition, lairage following extreme cold transport ($T < -8^{o}C$) could exacerbate the condition for some birds. Furthermore, it was shown that cold winter transportation could result in significantly higher live shrink (4% for temperatures below freezing compared to 1.4% for controls) and a substantial economic loss to the industry.

Results of this study further revealed the significant effect of cold exposure during winter transportation on quality attributes of breast and thigh meat by causing significant increases in WHC and WBC of breast meat and significant increase in pH_u and darkness of breast and thigh meat. The darker meat might not be acceptable to the fresh meat consumers and, therefore, may causes loss to the meat industry. As a result of cold exposure very high incidences of DFD breast (>50%) and thigh (> 80%) meat was reported in this study. The high incidence of DFD at exposure temperatures below -11°C reported here may, in large part, be related to the individual housing of birds during treatment and their inability to huddle together in order to maintain their CBT at acceptable levels and has not been reported elsewhere. Nevertheless, commercial broiler

transportation during Canadian winters can present exposure to temperatures below -11ºC as showed in the first phase of this research study.

The current study also highlighted the differences between breast and thigh muscles in response to cold-stress, with thigh oxidative muscle being more greatly affected by cold winter transportation when compared to breast glycolytic muscle. Biochemical changes in thigh meat were clearly explained based on energy reserves at slaughter, which was highly correlated to subsequent quality of this meat. While, it was shown that breast muscle post-mortem metabolism was very complicated and could not be clearly explained based on concentrations of muscle metabolites alone, which suggests that further investigation of DFD development in broiler breast meat is warranted.

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